HE 20.3160:68/1
Med.
507-N-9

[redacted]

CANCER TREATMENT REPORTS

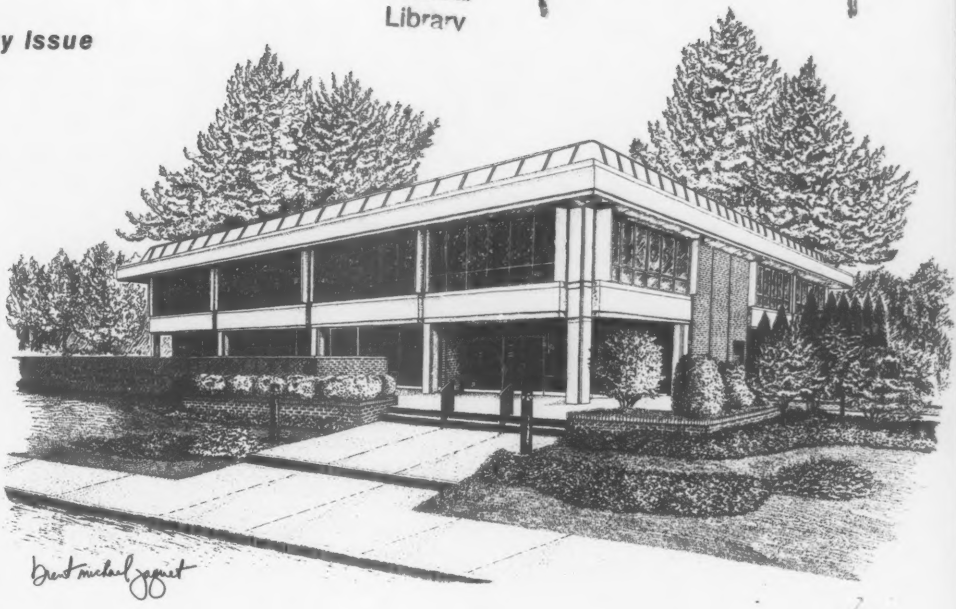
MEDICAL LIBRARY
RC
271
.C5
C265
v.68
no.1

SERIAL

THE UNIVERSITY OF MICHIGAN
FEB 28 1984
MEDICAL LIBRARY

The University
of Michigan
Medical
Library

25th Anniversary Issue
January 1984
Volume 68
Number 1
Pages 1-356



UNIVERSITY OF MICHIGAN
LIBRARIES

FEB 15 1984

DEPOSITED BY THE
UNITED STATES OF AMERICA

U.S.Department of Health
and Human Services
Public Health Service
National Institutes of Health

The 25th anniversary issue introduces a new cover for *Cancer Treatment Reports.* The pen and ink drawing by Brent Jaquet depicts the R. A. Bloch International Cancer Information Center, which was recently donated to the NCI and dedicated on October 2, 1983 (see Dr. Brandt's speech reprinted on page 5 of this issue). The editorial offices of the Cancer Treatment Reports Section, a component of the Scientific Information Branch, Office of International Affairs, OD, NCI, are now located in this building. The cover design is by the Medical Arts and Photography Branch of NIH.

CANCER
TREATMENT REPORTS

National Cancer Institute, Vincent T. DeVita, Jr, ***Director***
Scientific Information Branch, Susan Molloy Hubbard, ***Chief***

Editorial Board

Bruce A. Chabner, ***Director***, Division of Cancer Treatment
Robert E. Wittes, ***Editor-In-Chief***

Associate Editors
Joseph Aisner
Lewis Aronow
Desmond Carney
Richard L. Cysyk
William D. DeWys
Ross C. Donehower
Daniel C. Ihde
Allen S. Lichter
Robert W. Makuch
David G. Poplack
Richard Simon
Frederick P. Smith

Advisory Board
Gianni Bonadonna
George P. Canellos
Richard H. Creech
Harvey M. Golomb
Robert L. Goodman
Stephen B. Howell
Randall K. Johnson
Irwin H. Krakoff
Brigid G. Leventhal
Robert B. Livingston
Albert F. LoBuglio
William L. McGuire
Franco M. Muggia
Carol S. Portlock
Sydney E. Salmon

Editorial Staff
Jean Griffin Baum, *Managing Editor*
Dee Kittner LeRoy, *Assistant Managing Editor*
Anne McCarthy, *Symposium Editor*
Elaine Price Beck, *Editor*
Maralyn Farber Berlin, *Editor*
Jana Johnston, *Editorial Assistant*
Anna D'Avella
Elaine Brown
Robin Young

Editorial Policy

The Editorial Board of *Cancer Treatment Reports* will consider manuscripts on original observations in laboratory and clinical research from all scientists. A manuscript is considered for publication with the understanding that it has not been published previously and is submitted exclusively to *Cancer Treatment Reports*. Opinions expressed by the authors are not necessarily those of the publisher or its editors. Scientific contributions in any of the following areas of investigation are solicited: surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

Submission of Manuscripts

Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, R. A. Bloch International Cancer Information Center, Bldg 82, Rm 235, National Institutes of Health, Bethesda, MD 20205 (301–496–6975). All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board. Prospective authors should consult the "Instructions to Authors," which appears on the second page of every issue.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402.

INSTRUCTIONS TO AUTHORS

Title Page

Includes complete title of the manuscript and the full name, affiliation, and address of each author on the title page. Indicate full name and address of author to whom correspondence and/or reprint requests are to be sent.

Summary

Begin the article with a short summary.

Text

Submit an original and 3 copies of the manuscript, typed double-spaced. Dot-matrix, computer-generated or italicized texts will not be accepted. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables

Submit each table on a separate page, typed double-spaced. Please use only standard abbreviations; otherwise use full words.

References

Submit references typed double-spaced. Follow the standard form of *Index Medicus*: author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, chapter title, book title, city and name of publisher, date of publication, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers published in meeting proceedings should include author; title of paper; title of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations

Photographs and graphs should be clear, glossy prints. If original art work is submitted, it should be done in black ink for good reproduction. Please send 2 copies of each print. Photocopies reproduce poorly. Color photographs cannot be used.

Nomenclature

Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names* or *The Merck Index* for proper nomenclature.

Brief Reports

Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary, figures are usually omitted.

Letters to the Editor

Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

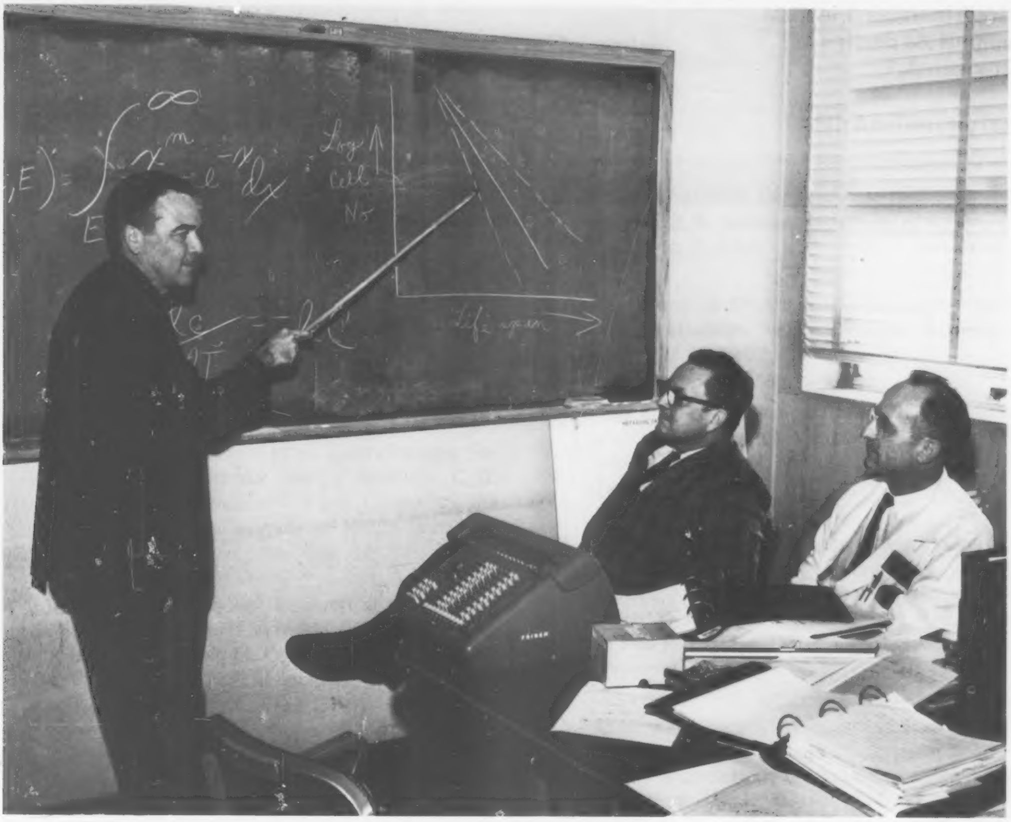
Clinical Trials Summaries

Manuscripts published in this section can be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen*, or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Reprints

Supplied free of charge, 100 reprints (20 reprints for Letter to the Editor) will be sent to the senior or specified author about 3 weeks after publication of the article.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1985.



Shown above (left to right) are William S. Wilcox (deceased), Howard E. Skipper, and Frank M. Schabel, Jr (deceased) in a photograph taken in 1964.

The 25th Anniversary Issue of *Cancer Treatment Reports* is dedicated to Dr. Schabel, who died August 30, 1983, in tribute to his outstanding achievements in chemotherapy research. He appears as co-author with Dr. Skipper in this issue in an article on Tumor Stem Cell Heterogeneity. Some comments from his colleague, Dr. John Montgomery, are given below.

Frank M. Schabel, Jr 1918-1983

I have been asked to make some comments about my relationship with Frank Schabel. That is not an easy task. I first met Frank 31 years ago when I started work at Southern Research Institute. Over the years, he became one of my closest friends as well as a respected scientific colleague. Frank was one of the most outstanding experimental chemotherapists in the world in both oncology and virology. Unfortunately, he had little time to teach—had he done so, there might not be the dearth of well-trained experimental chemotherapists that we have today.

Frank Schabel had no hobbies or outside interests except his family—his wife and two sons, and his mother, whom he visited regularly, even though it was not always easy for him to make that long trip back to Montana. His work was his hobby—and such enthusiasm! He believed deeply in the promise of chemotherapy and was always impatient at our rate of progress, but he was never discouraged. Most of us have good days and bad days, but not Frank. To him, every day was another opportunity to expand our knowledge, to push on and make a new contribution. There were no real failures, only temporary setbacks. He inspired all of us to dream dreams, and then to work like the devil to make those dreams come true.

Outside Southern Research Institute, Frank Schabel had many close friends and admirers. It is not only the regard for his outstanding scientific contributions, but his humor and the warmth of his relationships with people—it was hard not to like him. It may be true that no one is indispensable, but Frank Schabel is irreplaceable.

John A. Montgomery
Director, Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama

Contents

Cancer Treatment Reports
Volume 68, Number 1
January 1984

25th Anniversary Issue

---

1 On Special Initiatives, Critics, and The National Cancer Program. **V. T. DeVita, Jr.** National Cancer Institute, Bethesda, Maryland

5 Address Presented at the Dedication of the R. A. Bloch International Cancer Information Center, Bethesda, Maryland, October 2, 1983. **E. N. Brandt, Jr.** US Department of Health and Human Services, Washington, DC

9 Origins and Development of Chemotherapy Research at the National Cancer Institute. **C. G. Zubrod.** Comprehensive Cancer Center for the State of Florida, University of Miami School of Medicine

21 Intramural Therapeutic Research at the National Cancer Institute, Department of Medicine: 1955–1965. **E. Frei, III.** Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts

31 Cancer Surgery: A Commentary. **B. Fisher.** University of Pittsburgh, Pennsylvania

43 Tumor Stem Cell Heterogeneity: Implications With Respect to Classification of Cancers by Chemotherapeutic Effect. **H. E. Skipper and F. M. Schabel, Jr.** Southern Research Institute, Birmingham, Alabama

63 The Preclinical New Drug Research Program of the National Cancer Institute. **J. S. Driscoll.** National Cancer Institute, Bethesda, Maryland

77 Clinical Development of Anticancer Agents—A National Cancer Institute Perspective. **S. Marsoni and R. Wittes.** National Cancer Institute, Bethesda, Maryland

87 Drug Resistance in Cancer. **G. A. Curt, N. J. Clendeninn, and B. A. Chabner.** National Cancer Institute, Bethesda, Maryland

101 Regional Cancer Chemotherapy. **W. D. Ensminger and J. W. Gyves.** University of Michigan Medical School, Upjohn Center for Clinical Pharmacology, Ann Arbor

117 Human Tumor Colony Assay and Chemosensitivity Testing. **S. E. Salmon.** University of Arizona Cancer Center, Tucson

127 Liposomes as Drug Carriers in Cancer Therapy. **J. N. Weinstein.** National Cancer Institute, Bethesda, Maryland

137 Antitumor Antibiotics and Low Molecular Weight Immunomodifiers of Microbial Origin. **H. Umezawa.** Institute of Microbial Chemistry, Tokyo, Japan

145 Role of Bone Marrow Transplantation in the Treatment of Hematologic Malignancies and Solid Tumors: Critical Review of Syngeneic, Autologous, and Allogeneic Transplants. **R. E. Champlin and R. P. Gale.** UCLA School of Medicine, Los Angeles, California

163 Antiemetic Therapy: A Review of Recent Studies and a Report of a Random Assignment Trial Comparing Metoclopramide With Delta-9-tetrahydrocannabinol. **R. J. Gralla, L. B. Tyson, L. A. Bordin, R. A. Clark, D. P. Kelsen, M. G. Kris, L. B. Kalman, and S. Groshen.** Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, New York

173 Advances in the Management of Cancer Pain. **R. Payne and K. M. Foley.** Cornell University Medical College and Memorial Sloan-Kettering Cancer Center, New York, New York

185 Importance of Prognostic Factors in Cancer Clinical Trials. **R. Simon.** National Cancer Institute, Bethesda, Maryland

193 Recent Concepts of Cancer Metastasis and Their Implications for Therapy. **I. J. Fidler.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

199 Induced Cell Differentiation in Cancer Therapy. **A. Bloch.** Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo

207 Current Condition and Prognosis of Tumor Immunotherapy: A Second Opinion. **M. J. Mastrangelo, D. Berd, and H. C. Maguire, Jr.** Fox Chase Cancer Center, Philadelphia, Pennsylvania

221 Biologicals and Biological Response Modifiers: Fourth Modality of Cancer Treatment. **R. K. Oldham.** National Cancer Institute, Frederick, Maryland

233 Adoptive Immunotherapy of Cancer: Accomplishments and Prospects. **S. A. Rosenberg.** National Cancer Institute, Bethesda, Maryland

257 Monoclonal Antitumor Antibodies in the Lymphatics. **J. N. Weinstein, M. A. Steller, D. G. Covell, O. D. Holton, III, A. M. Keenan, S. M. Sieber, and R. J. Parker.** National Cancer Institute, Bethesda, Maryland

265 Role of Receptors in Mediating Steroid Hormone Effects in Human Breast Cancer. **M. E. Lippman and A. Kasid.** National Cancer Institute, Bethesda, Maryland

281 Potential Use of Analogs of Luteinizing Hormone-Releasing Hormones in the Treatment of Hormone-Sensitive Neoplasms. **A. V. Schally, T. W. Redding, and A. M. Comaru-Schally.** Veterans Administration Medical Center and Tulane Medical School, New Orleans, Louisiana

291 Promise of Radiosensitizers and Radioprotectors in the Treatment of Human Cancer. **T. L. Phillips and T. H. Wasserman.** University of California, San Francisco

303 Particle-Beam Radiation Therapy 1983: Evaluation and Recommendations. **M. P. Richter and M. M. Kligerman.** University of Pennsylvania and the Fox Chase Cancer Center, Philadelphia

309 Hyperthermia as a Clinical Treatment Modality. **T. V. Samulski, E. R. Lee, and G. M. Hahn.** Stanford University, California

317 Diagnosis of and Therapy for Solid Tumors With Radiolabeled Antibodies and Immune Fragments. **J. A. Carrasquillo, K. A. Krohn, P. Beaumier, R. W. McGuffin, J. P. Brown, K. E. Hellstrom, I. Hellstrom, and S. M. Larson.** Veterans Administration Medical Center, Seattle, Washington

329 *Cancer Treatment Reports* 1959–1983: Background Review and Tabular Compilation of Most Cited Articles. **E. M. Sloane, J. G. Baum, S. M. Hubbard, and R. E. Wittes.** National Cancer Institute, Bethesda, Maryland

339 APPENDIX

  339 Contrasting Viewpoints on Cancer Development: The Wooldridge and Richardson Reports. **V. T. DeVita, Jr.** National Cancer Institute, Bethesda, Maryland

  340 A Review of the Report of the 1966 Cancer Chemotherapy Collaborative Program Review Committee. **A. P. Richardson.** Emory University School of Medicine, Atlanta, Georgia

  343 Report of the Cancer Chemotherapy Collaborative Program Review Committee: The Richardson Report.

On Special Initiatives, Critics, and The National Cancer Program

Vincent T. DeVita, Jr[1,*]

Happy anniversary, *Cancer Treatment Reports*. Your 25 years have chronicled much change in cancer treatment, especially the addition of chemotherapy to the armamentarium of cancer medicine. Successful cancer chemotherapy resulted from the Institute's oft-criticized special initiative in cancer drug development. This is a fitting opportunity, then, to comment on special initiatives, their critics, and the National Cancer Program.

The National Cancer Institute (NCI) was established as a separate entity by an act of the Congress in 1937 because cancer as a disease entity was then, and has remained, the No. 1 health concern of the American people. The Institute is different from other institutes of the National Institutes of Health (NIH) in important ways. Some differences are unique to cancer, the disease, as it is viewed by the public; others are due to special initiatives which afforded the Institute opportunities to capitalize on developments in research and medicine.

Three of these special initiatives changed NCI even prior to the passage of the National Cancer Act in 1971. All three initiatives were characterized by turbulent births and stormy childhoods. They all, I am pleased to report, are enjoying healthy and productive adult lives. The Drug Development Program established in 1955 was first, the Special Virus Leukemia Program established in 1964 was second, and the Chemical Testing Program, begun around 1968, was third. The first two programs were special congressional mandates, while the third was an attempt to build on the model of the previous two. All three were controversial because they implied targeting of research and they used the contract as a funding instrument. Targeting implies a foreknowledge of where you are going, which is the very antithesis of the research process. It is for this reason that I prefer the more accurate terminology, "special initiatives," which implies only emphasis related to opportunity rather than clearly defined targets. It is not necessary to dwell on the use of contracts in this essay since the implication in the use of contracts has been discussed recently in detail elsewhere (1). Suffice it to say only that, when NIH programs are controversial in concept and an unconventional mechanism for support is used, the focus of criticism can shift from the intent of the program to the mechanism of support. Such shifts tend to cloud any discussion of the basic merit of the program. During the past two decades this occurred with all three of the special initiatives mentioned above when the use of contracts became the major issue.

Dr. Gordon Zubrod, later in this issue, will review the origins of the chemotherapy program much better than I ever could. He will undoubtedly say the program has been a success. The program can add to its tally the cure of over a dozen cancers not curable prior to the availability of drugs and the improvement in survival for an increasing number of common cancers. Critics of this program (and there are still critics) point out that a large number of common cancers are still unresponsive to drugs. This is certainly true. However, these criticisms tend to ignore the overriding importance of the experiment in cancer chemotherapy conducted between 1955 and 1970: to test the hypothesis that anticancer drugs, like antibiotics and antimalarials before them, could, by themselves, cure metastatic cancer. If the answer were "yes" (which turned out to be the case), then its impact on our understanding of the biology of the cancer cell would be profound. It would also mean that anticancer drugs could be utilized in a wide variety of more favorable circumstances in concert with surgery and radiotherapy. This has happened since 1970. The Drug Development Program was established when Congress overrode the rejection by the National Advisory Cancer Council of the concept of a concerted cancer drug development effort. This sequence of events angered critics of cancer drug development and negatively influenced their willingness to objectively measure progress in cancer chemotherapy in the years that ensued. In response, they demanded that only instant and total success could justify this investment, which they considered to be a dislocation of the normal scientific process. Instead, what has happened is what usually happens in medicine—slow, steady, but significant progress.

What Dr. Zubrod might *not* say is that as much energy was expended in the scientific community to stop cancer drug development as was exerted to get it off the ground.

---

[1] Director, National Cancer Institute, Bethesda, MD.

[*] *Reprint requests to:* Vincent T. DeVita, Jr, MD, Director, National Cancer Institute, Bldg 31, Rm 11A52, National Institutes of Health, Bethesda, MD 20205.

The self-fulfilling prophecy was the backbone of the critics' logic: after all, metastatic cancer was an incurable disease; since it was an incurable disease, why treat it? But, without any treatment, it is indeed incurable. Drugs are toxic; why make people with incurable diseases even sicker than they are already? In the early 1960s this was the standing argument faced by anyone who sought to develop protocols with curative intent. Unfortunately, this self-fulfilling prophecy lies in wait to pounce on every new attempt to improve the therapy of a previously untreatable disease. Between 1955 and 1970, a relative handful of investigators staked their careers and reputations on breaking that Gordian knot.

What Dr. Zubrod will almost certainly *not* say is that one person meant the difference between success and oblivion for the cancer drug development program and cancer chemotherapy—and that person was, in fact, Gordon Zubrod. In addition to helping implement new concepts for the treatment of cancer with drugs, he bore the brunt of the criticism in and out of the NIH. Now cancer chemotherapy and the specialty of medical oncology are established side by side with surgery and radiotherapy as primary forms of cancer treatment. Now there are thousands of medical oncologists practicing in communities all over America. Now the pharmaceutical industry, absent in the early days of cancer drug development, is heavily involved in developing cancer drugs. Now, thanks to the biologic revolution, specific targets for anticancer drugs are coming into clearer focus.

The second special initiative followed on the heels of the Cancer Drug Development Program. This was the Special Virus Leukemia Program which in 1969 became the Special Virus Cancer Program. Like drug development, this initiative came from the Congress via enthusiasts in the research community and, like drug development, it faced persistent negative reactions from the scientific community. It is now, for example, referred to as the Biological Carcinogenesis Program, a name it adopted in 1979 to take cover from critics of the resources expended on viral oncology. Again, the focus of criticism was on the problems in contracting and the perceived dislocation of what was considered the normal research process, rather than on the scientific merit of the program. Although the criticism obscured the scientific progress in this program for a number of years, viral oncology can come out of the closet now. The exciting story of viruses and cancer was accelerated with the discovery of the enzyme, reverse transcriptase, and the homology between retroviral oncogenes and the equivalent transforming genes in human cancer cells. Now the molecular biology of oncogenes promises to lead us to an understanding of the very switches that result in the phenotypic cellular change we know as cancer. The initial goal of this program was to find human cancer viruses analogous to the viruses that caused cancer in rodents, cows, and chickens—and these early attempts failed. What evolved instead was a profound understanding of the genetics of cancer viruses that needed only the tremendous technologic revolution of the 1970s to allow it to blossom into the most exciting scientific story of the 1980s. In the end, it gave us what it set out to look for as well—viruses that cause human cancers.

The third special initiative is less exciting but nonetheless important. In the 1960s the Institute began to develop a formal testing program in rodents to screen chemicals for potential carcinogenicity, the Bioassay Program. Like the Drug Development Program and the Special Virus Cancer Program before it, the Bioassay Program was implemented with the mechanism of contracts and it was troubled by the logistic difficulties in establishing both sound scientific methodology and physical facilities for handling large numbers of rodents exposed to carcinogens over long periods of time. Nonetheless, it provided the foundation for the collection of the only data which allow Congress and regulatory agencies to buttress laws and regulations with scientific fact and logic. It also provided the data base which allows us to evaluate the use of simpler tests that will ultimately replace testing in rodents to identify the carcinogenic potential of environmental agents. It constituted, as well, the foundation for the broader toxicologic testing of chemicals entering the environment and, with this, its mission also broadened. In 1982 the NCI transferred this 47.9 million-dollar program, along with some 95 staff who managed it, to the National Institute of Environmental Health Sciences (NIEHS) since its broader mission more closely matched that of NIEHS.

By 1970 these and other initiatives had produced pent-up energy in the field of cancer research, a certain promise that a critical mass of information was at hand to justify an even grander initiative. This energy led to the creation of the Yarborough Commission, which in turn concluded that a critical mass of *opportunity* did exist that warranted a complete program to provide all of the necessary tools to accelerate the attack on cancer (2). Passage of the National Cancer Act followed in 1971, and the flow of resources increased between 1972 and 1976, before the increases levelled off between 1978 and 1982.

The greatest portion of resources supplied to the Cancer Program appropriately went to support basic research. Between 1972 and 1983, NCI invested over one-half of its budget to support basic research through traditional grant mechanisms. In large measure, this investment can be given a significant share of the credit for the biologic revolution we are witnessing today. Criticism of the Cancer Program was swift in coming, however, and just as demanding of instant and total success as the price for yet another special cancer initiative. This was because explicit in the Cancer Act was the provision of resources and instruments to apply the results of basic research. The architects of the National Cancer Program felt that since basic research was supported with public funds, without a firm commitment to the swift application of the results of basic research, public interest in supporting research would falter. Then, rather than having more resources for basic research, the result would be a diminution of support.

It is for these reasons that special tools were given to the Institute. These tools were: (*a*) an expanded cancer centers program, to clone the concept of the National Cancer Program throughout the country; (*b*) an expanded clinical trials program, to test new approaches to treatment and prevention in those who needed it most—persons who had or were subject to cancer (The expanded clinical trials program allowed adjuvant chemotherapy to come under proper scrutiny. Dr. Fisher will, later in this issue, chronicle the development of such trials in breast cancer, trials which he pioneered.); (*c*) the Cancer Control Program, a program to disseminate state-of-the-art information to the professional community; and (*d*) information-gathering and disseminating programs, NCI's Office of Cancer Communications for the public and the International Cancer Research Data Bank for physicians and scientists. The latter made possible the final link between the bench and the bedside in cancer treatment—the new PDQ (Physician Data Query) system, a computerized data base that indicates who is doing what and where in cancer treatment and provides state-of-the-art information on treatment of all cancers (3). The importance of this latter program is underscored by the comments made in this issue by Dr. Edward Brandt, Assistant Secretary for Health of the Department of Health and Human Services. Like *Cancer Treatment Reports* and the *Journal of the National Cancer Institute*, as well as other parts of NCI's Scientific Information Branch, this new electronic communication system will be housed in the R. A. Bloch International Cancer Information Center depicted on the cover of this issue.

All of these programs were controversial, but none as controversial as the Cancer Control Program. Many of the architects of the National Cancer Program were the same persons who had urged Congress to support the other special initiatives before the passage of the Cancer Act. It got off to a shaky start. Because of the criticism of special initiatives, especially against attempts to apply the results of basic research, the Cancer Control Program was deliberately created as a separate budget line item unavailable for support of basic research. Also, the Cancer Control Program (by NCI's choice at that time) excluded the use of its funds for any research. The definition of control was unclear, and the tools to implement the concept, had they been clearly defined, were not yet developed. In retrospect, the early emphasis should have been on the development of the concepts and tools rather than what was done—the development of a plethora of demonstration projects, primarily in treatment. This provided even more grist for the critics' mill. Now I am pleased to report that the concepts of cancer control have been clearly formulated, and research and cancer control are now synonymous, although its research takes a different shape. The sequence of phases of cancer control have been clearly defined, and programs such as the Cancer Control Research Units, Community Clinical Oncology Program, Cancer Control Science Program, and PDQ are in place to link the concepts to a program structure for implementation. The Cancer Control Program has also been combined in the same division with the Cancer Centers Program since the latter, in addition to providing an environment in which research can flourish, is an invaluable instrument for implementing the programs of the former. The Institute's support of basic research in epidemiology, diet and nutrition, viral oncology, chemical carcinogenesis, and immunology has now provided the successful framework for clinical trials in cancer prevention and the Cancer Control Program is now serving as an effector arm in cancer prevention rather than as a demonstration program in cancer treatment. The Cancer Control Program is, I believe, now constructed as it was originally envisioned by the architects of the National Cancer Program. While it remains a special line item in the budget, with all the inflexibility implicit in this budget mechanism, it is no longer on uncertain turf (4,5).

All research is built on previous knowledge, but the term "revolution" implies the rapid acceleration of change. I stated earlier that the National Cancer Program can take a prodigious share of the credit for kicking off the biologic revolution. The biologic revolution began to accelerate in the early 1970s. It has taken two lines of development: the first is advances in technology and the second is accelerated discoveries as a result of the use of these technologies. My own feeling is that the realization of the value of restriction enzymes as a tool in molecular biology can be viewed as a convenient recent take-off point, followed quickly by the discovery of the reverse flow of genetic information with the enzyme, reverse transcriptase, a discovery which, in addition to its purely scientific value, has helped spawn a whole biotechnology industry. This was followed by DNA recombinant technology, rapid DNA sequencing, and hybridomas and monoclonal antibodies. These technologic advances resulted in our understanding of split genes, a revolution in itself, the complex rearrangement of immunoglobulin genes, the whole oncogene story, the sure and steady mapping of the human genome, and our ability to fingerprint cell surfaces to such an extent that we are now beginning to understand the differences between cell membranes of normal and cancerous cells, and metastatic potential as a phenomenon separate from malignant transformation. The potential of all this information lies in the development of new and more specific ways of both preventing and treating cancer.

It is, in my view, without doubt that the National Cancer Program has been an astonishing success—an investment that has already paid for itself while the best is yet to come. The management challenge confronting NIH—not just NCI—is to maintain the flexibility to continue the strategy of rapidly shifting resources to high-priority programs that offer the most promise, regardless of the trauma such shifts cause to the structure of the research apparatus or, for that matter, to the practice of medicine. This may seem a callous way of framing this imperative, but we should, it seems to me, bear in mind that the NIH and the National Cancer Program were not established as an *end*—but rather, as a *means* to an end. We are in the midst of a biologic revolution which, in my view, will shake the very foundations of cancer medicine. As *Cancer Treatment Reports* celebrates its 25th anniversary in a new home, it is appropriate to ponder whether we are equal to the challenge.

REFERENCES

1. DEVITA VT. The governance of science at the National Cancer Institute: a perspective on misperceptions. Cancer Res 43:1891-1898, 1983.
2. National Program for the Conquest of Cancer. Report of the National Panel of Consultants on the Conquest of Cancer, authorized by S. Res. 376. Prepared for the Committee on Labor and Public Welfare, 92nd Congress.
3. DEVITA VT. PDQ: bringing the latest in cancer diagnosis and treatment to the community physician. Your Patient & Cancer, 88-95, Sept 1983.
4. GREENWALD P. New directions in cancer control. John Hopkins Med J 151:209-213, 1982.
5. GREENWALD P. Cancer control research programs of the National Cancer Institute. *In* Progress in Cancer Control IV: Research in the Cancer Center. New York, Alan Liss, Inc, 1983, pp 19-27.

Address Presented at the Dedication of the R. A. Bloch International Cancer Information Center, Bethesda, Maryland, October 2, 1983

Edward N. Brandt, Jr[1,*]

Mr. and Mrs. Bloch, Dr. Carter, Dr. Wyngaarden, Dr. DeVita, distinguished guests, ladies and gentlemen.

It's a special pleasure for me to be here today to join you in the dedication of this extraordinary building. This ceremony is an important event in mankind's long and heart-struck battle against cancer.

What we are doing today is opening up the archive of medical research as it has never been opened before. We are making it possible for the worlds of research and of clinical practice to come together . . . to exchange information and experience . . . and to insure that the benefits of that exchange accrue to cancer patients and the population in general.

But we're doing something else today as well. We're together at this ceremony to pay honor to a fundamental American idea—the idea that private individuals can change and improve the course of history . . . that private initiative and capital are integral parts of the American social fabric, contributing in a way every bit as significant as the contributions of government.

But it's difficult to honor an *idea*. We can say we want to do that and we might even succeed. But this afternoon we can see the reality of that idea in the men and women who grace this platform and this audience.

And I want to give particular honor to one private citizen here today, Mr. Richard A. Bloch, whose vision and dedication have made this building possible. . . he made a dream come true. His first and major contribution to the fund for this building was a challenge to others in the private sector who are also committed to battling the scourge of cancer.

And they came through. Together, they provided the $1.4 million needed to purchase this handsome building here, virtually on the perimeter of the NIH campus.

But this building is to be known—quite appropriately—as the "Bloch Center" because the initial infusion of energy, interest, commitment, and money came from Mr. Richard Bloch. This building and the humming information traffic inside it are to be reflections of Richard Bloch's depth of feeling for the hundreds of thousands of individual cancer patients around the country whose lives depend on the generation and application of new knowledge . . . His grasp of the seriousness of the deficit of information that exists across so many areas of clinical oncology . . . And his understanding—gained from profound personal experience—of the needs of the attending physician, who carries the fight—bedside-to-bedside—in communities right across this country and, indeed, around the world. I think it is fair to say that Mr. Bloch and his wife, Annette, challenged the imaginations and the giving capacities of other private citizens. They set a standard to which they asked others to aspire. The response was more than just "heartening." I think you can say it was "life-saving."

To recognize the contributors to the Richard A. Bloch Center is to recognize the men and women in this country who *act* as well as *care*. They are: Richard and Annette Bloch; Mr. and Mrs. Kenneth J. Jonsson; Mr. Alan I. Kay; Abe and Irene Pollin; Mr. and Mrs. James A. Sheehan; and The Wald Foundation in memory of Alex Wald. These men and women—and other contributors as well—have proved themselves to be outstanding citizens of this country and caring members of the human family.

But it would be a grave oversight if I did not say a few words about the *need* for this building and the kind of work that will be going on inside. And here I have to be very careful and not get carried away.

One of my first appointments at the University of Oklahoma Medical Center, some 20 years ago, was as the Director of the Medical Research Computer Center for the Department of Preventive Medicine and Public Health.

So I'm here today not only in my official capacity as your Assistant Secretary for Health, but also in my own personal capacity as a believer in what technology can do for the health of the American people.

Much has happened in the computing sciences during those 20 years. I began my career as a baby-sitter for the big IBM 1401, which has since become something of a museum piece. Then, in the mid-60s, the IBM 360 came

---

[1] Dr. Brandt is the Assistant Secretary for Health, US Department of Health and Human Services (DHHS), Washington, DC.

[*] *Reprint requests to:* Dr. Edward N. Brandt, Jr, Assistant Secretary for Health, DHHS, 200 Independence Ave, SW, Rm 716G, Washington, DC 20201.

along and changed the future history of the electronic computer. Next have come chips and micro-chips to further revolutionize the field of computing. And today, of course, we have the personal computers—the "PCs"—that fit on your desk or in your briefcase.

And it so happens that one of the fastest growing segments within the field of computers is the personal computer for physicians. I am told that a new journal will be appearing this month titled *"The Computing Physician."* It will be followed by at least three more covering the same general area . . . that is, helping physicians make better use of the new personal computers.

The American Medical Association is now working out the details for adapting the GTE Corporation's "TELENET" to transmit medical and health data between the AMA's own large reservoir of information and the individual computing physician anywhere in the country.

That is, of course, the concept also being realized so dramatically at this new center. This building will house a precedent-setting computerized data base accessible to physicians and medical institutions around the country and around the world.

And I think the record will show that the National Cancer Institute has consistently stayed close to these developments in information technology in order to find more effective and efficient ways to pull together the many different communities of interest in the vital and rapidly expanding field of cancer research.

Even as we've been learning so much about information technology and computers during the past 20 years, we've also been learning a great deal about "cancer." From 1962 through 1982 the National Cancer Institute awarded a total of $7.2 billion in grants and contracts for cancer R&D. The major portion of that money—$6.1 billion—was awarded just in the past 10 years, as a result of the landmark National Cancer Act of 1971.

To that sum I would add several millions more in funds that have been spent by other agencies of the public health service and other institutes at NIH in pursuit of the causes and treatments of cancer.

In the private sector, organizations like the American Cancer Society and industrial sponsors like the pharmaceutical manufacturers have also invested many hundreds of millions of dollars in basic and applied research.

What has been the result of this enormous investment? Actually, there have been *many* important results which, taken together, have meant the return of health and life to millions of Americans. But for our purposes today, I would cite the explosion in cancer research information that has occurred primarily because of that $7.2 billion investment by the Cancer Institute.

Currently some 3000 journals regularly publish in excess of 50,000 articles a year, presenting to the medical profession new results from cancer research. This material is invaluable. Much of it can be life-saving in day-to-day medical practice. But can the average bedside practitioner handle that kind of volume? No . . . It's just too much.

And that has to be a major concern to us. We know for a fact that 85 percent of all cancer patients remain close to their homes, to their families, and to their friends . . . close to the people who love them and care about them, the people who will ease them along either the arduous return journey to health and life . . . or the more profoundly difficult journey to death.

We're now talking about an estimated 3.5 million Americans of all ages who have cancer—or who *did* have it sometime during the past 10 years—who are still alive and who prefer to stay in their home communities in the care of local physicians.

Those physicians, through their routine practice, ought to be able to benefit from research results coming into the literature or should themselves—with the informed consent of their patients—be able to make important contributions *to* that same literature. But to do either or both, physicians—even in the remotest areas—must have easy access to that literature.

I think I should add—because it's only fair—that it's been a part of this country's national health policy to encourage new physicians to practice in smaller communities, rather than crowd into a few big cities that are already well supplied with practitioners of every specialty. So, ever since 1971, when the program began, the US Public Health Service, with the support of the President and the Congress, has recruited young physicians, dentists, nurses, and other professionals to serve in remote and medically *un*served and *under*served areas.

It is also national policy to make sure that those same young physicians practice the very best medicine possible . . . that both they and their patients can take advantage of the same information that's available to physicians and hospital staffs in large metropolitan areas.

So we have told these young people that, if they choose to sink roots in those areas, set up their own practices, and raise families, we will make sure they nevertheless remain in touch with mainstream American medicine. It's important for them . . . and it's absolutely vital for their patients.

The Bloch Center is, among other things, another way in which the Public Health Service can keep its promise to those fine young professionals caring for the health needs of Americans in rural and urban ghettos . . . on Indian reservations . . . in small mining and farming communities . . . and in other isolated corners of our nation.

We know that cancer is an equal opportunity destroyer . . . it makes no distinctions as to its victim's race, color, creed, national origin, social or economic status, or place of residence. And, therefore, *neither can we*.

Hence, through the use of "PDQ" and the forthcoming "CANCERINFO" file and the computerized protocol summaries, physicians anywhere in the country will be literally just "a byte away" from the latest data available on specific forms of cancer at specific body sites of patients with specific personal histories.

During these few moments I've spent at the rostrum, I realize that I've focused mainly on physicians and cancer *patients*. And it is certainly true that systems like "PDQ" and "CANCERINFO" and the new "Community Clinical Oncology Program" are focused entirely upon the identification and treatment of the disease. We talk of the "Cancer Treatment Network" and sometimes we leave it at that . . . not looking at the issue from another side. And I want to mention that side right now, because I believe it also will have a place in the Bloch Center information archive.

I'm talking about the prevention of cancer.

As with the other two major killers of our people—heart disease and stroke—we don't really know what cancer is. Yet, science has deduced that certain events or circumstances can cause it.

The best example I can offer—but one of the worst examples of personal health behavior—is smoking. We estimate that smoking will account for about 25 percent of the new cases of cancer to be diagnosed and treated this year. That will come to about 210,000 men and women who have smoked and now have cancer.

And I can tell you that medical science knows without a shadow of a doubt that cigarette smoking is the *major single cause of cancer mortality* in the United States. This year, close to 130,000 Americans will die prematurely from smoking-related cancers . . . about 95,000 from lung cancer, 8000 from mouth and throat cancers, and 7000 from bladder and kidney cancers.

Some of these smoking-related cancers have as good or better 5-year survival rates than the national overall rate, which now stands at 46 percent. However, other smoking-related cancers have terrible survival rates. Here are just two examples:

We estimate that smoking is a factor in about 30 percent of all deaths from *pancreatic cancer*, or about 6500 deaths a year. The 5-year survival rate for pancreatic cancer is less than 1 chance in 10.

Cancer of the esophagus is even worse. Only 4 percent of its victims survive for 5 years or more. As a matter of fact, most patients die within 6 months after the onset of the disease. And we estimate that *over half the number of deaths from cancer of the esophagus are smoking-related*.

Along with everyone here today, I am encouraged by the steady success we are making in the diagnosis and treatment of cancer. And even though the number of new cancer patients rises with each successive year, we are also seeing a persistent rise in the 5-year survival rate.

But even more encouraging would be a turnaround and decline in the patient totals. And the only way that can come about, in my judgment, would be if 52 million Americans looked themselves in the mirror and said, "All right, I choose life not death . . . I'll give up smoking right now." And then they really do it.

Before too long, that single change in personal behavior or "lifestyle" would reduce the number of cancer deaths by more than 100,000 a year, something that can't be done today by any single treatment method or combination of treatment methods.

That's why the Public Health Service has made disease prevention and health promotion among the top priorities in our overall health policies. We feel the pay-off to individual Americans will be the best to be wished: good health and long life. But to get that, they will have to adopt a number of common sense rules of personal behavior, such as . . .

Don't smoke . . .

Maintain a healthy, balanced diet . . .

Exercise regularly . . .

Stop the abuse of alcohol and other drugs . . .

And control the way they respond to stress.

These are some of the simple and painless rules that we must apply to ourselves. To the extent that we do, we achieve our biggest victories over the major killers of our people: heart disease, stroke, and cancer.

But we know that this planet will turn many, many more times on its axis, before mankind will do those things that are to its benefit. So in the meantime, we're going to need every advantage we have to fight disease, disability, and death.

The Richard A. Bloch International Cancer Information Center is just such an advantage. To Richard and Annette Bloch, to all the contributors, and to the cancer research community, I extend my sincere thanks for bringing us to this hour.

Origins and Development of Chemotherapy Research at the National Cancer Institute

C. Gordon Zubrod[1,2,*]

It is an honor to contribute to this commemoration of the 25th anniversary of *Cancer Treatment Reports*. The history of chemotherapy at the National Cancer Institute (NCI) was last recorded in this journal in 1966 (1). The purpose of this paper is to identify and discuss the events implied in the title, tracing the evolution of chemotherapy research from its first faint stirrings at NCI in the 1930s to the acceptance of chemotherapy as standard treatment for a number of tumors and its inclusion in the broader objective of the Division of Cancer Treatment in 1972.

NCI Chemotherapy Research Prior to Opening of the Clinical Center (2)

Research in the chemotherapy of cancer at NCI had its beginnings about 1935 while the Institute was still in utero in Harvard. The US Public Health Service had created the Office of Field Investigations in Cancer under Dr. Joseph W. Schereschewsky in 1927, and it remained in Cambridge, MA, until 1939, when it was moved to Bethesda, MD, as part of the newly formed NCI under the directorship of Dr. Carl Voegtlin. The late Dr. Murray Shear joined Schereschewsky's group at Cambridge in the early 1930s and began studies on chemical carcinogenesis. In the mid-1930s he became intrigued by the effects of Coley's bacterial toxins on human sarcomas and turned to their study in animal tumors. He isolated a polysaccharide from *Serratia marcescens* which produced hemorrhage in S37 tumors in mice and organized a clinical group to try the polysaccharide in patients. The group consisted of Drs. Reimann and Oakey at Lankenau Hospital and Dr. A. L. Halloman of the US Public Health Service. Drs. Austin Brues and Rulon Rawson at Harvard also worked with Shear's materials. The polysaccharide brought about tumor regressions but gave rise to severe side effects, and it never received wide study.

While still at Cambridge, Shear had recruited Drs. Jonathan Hartwell and Joseph Leiter. The group continued work on carcinogenesis but they had a growing interest in the potentials of chemotherapy. In about 1945, a general screening program was established at NCI in Bethesda using the S37 tumor. In the next 5 years, 3000 chemicals and several hundred plant extracts were screened. There was also a limited synthesis program for analogs of two known active agents, arsenic and colchicine. The only agents clinically studied besides the polysaccharides were α-peltatin derived from podophyllin (mandrake) and tri-methyl colchicinic acid.

NCI had set up several satellite clinical operations. Prior to World War II, the NCI developed a tumor clinic at the Public Health Service Hospital in Baltimore, MD, under Dr. John Wirth. Its interactions with the scientists at NCI were limited until Dr. Abraham Goldin joined NCI in 1949 and was stationed at Baltimore awaiting the expanded resources in the Clinical Center. With Drs. Ezra Greenspan, Jacob Colsky, Emmanuel Schoenbach, John Venditti, Stuart Humphreys, and Paul Condit, an active chemotherapy group evolved. Their activities were notable mainly for the careful experimental drug studies that later became Goldin's trademark, but a beginning was also made in pharmacology and clinical trials. Goldin took advantage of the discovery of L1210 leukemia by Dr. Lloyd Law, NCI's great biologist. Law made a number of drug studies, especially with mercaptopurine, and Goldin later developed the L1210 model into a superb quantitative tool for studying antileukemia drugs. A second clinical satellite was initiated by Dr. Michael Shimkin, one of the original research fellows at Cambridge. In 1947, on returning from army service, Shimkin established laboratories and a 15-bed unit at the University of California in San Francisco. Drs. Howard Bierman, Jesse Steinfeld, Laurens White, and Nicholas Petrakis served in this unit. Clinical trials were undertaken with the new drugs then appearing and the first hypophysectomy for breast cancer was performed. Dr. Roy Hertz established a

---

[1] Comprehensive Cancer Center for the State of Florida, University of Miami School of Medicine.

[2] I thank Drs. Nathaniel Berlin, Emil Frei, Howard Skipper, J. Palmer Saunders, Charles Vogel, and the late Frank Schabel for reviewing my manuscript. They kept me honest about the facts and made many helpful suggestions. I am particularly indebted to Dr. Saunders for his kind permission to quote from a letter he sent me concerning the history of study sections and the CCIRC.

[*] *Reprint requests to:* C. Gordon Zubrod, MD, University of Miami School of Medicine, PO Box 016960 (D8-4), Miami, FL 33101.

third clinical unit at George Washington University Medical School having both laboratories and clinical resources for endocrinologic studies. Hertz was interested in nutritional influences on the endocrine organs and their tumors. Drs. William Tullner, Josiah Brown, and Monte Greer were members of this unit. When the Clinical Center opened, Hertz transferred his entire unit to Bethesda. Goldin, Venditti, Humphreys, and later Paul Condit moved from Baltimore to the Clinical Center, but the clinicians left for other institutions. The Baltimore-NCI relationship continued at a modest level, and years later expanded into a directly run NCI unit. The San Francisco unit was discontinued in 1954, and Drs. Shimkin, Steinfeld, and White moved to Bethesda.

It was slightly less than 20 years between Shear's first interest in the chemotherapy of cancer in Boston and the opening of the Clinical Center. This period had served to develop concepts and to interest scientists at NCI in cancer chemotherapy. Beginning experience had been gained in drug development including synthesis, extraction of natural products, biology of hormones, screening, use of biostatistics in experimental design, toxicology, pharmacology, and organization of clinical trials. The opening of the Clinical Center provided a splendid opportunity for growth of chemotherapy research. In the next 20 years, NCI became a dominant force in the studies that led to the first drug cures of cancer.

Clinical Chemotherapy at Last (1-3)

The Clinical Center opened in 1953, and Roy Hertz admitted the first patient, a person who had disseminated prostatic cancer. As a hospital, the Clinical Center did not have the luxury of polishing its operations over many decades. With six different Institutes involved, each with its own needs and concepts of patient care, there were problems in the overall functioning of a large hospital. Despite these concerns, the efforts of many fine physicians were successful, and the Clinical Center quickly became a viable place to care for the sick. Dr. J. Roderick Heller was Director of NCI, and Dr. G. Burroughs (Bo) Mider, a neurosurgeon turned pathologist and a man of great vision and wisdom, was Associate Director for Intramural Research. He carried the brunt of the early planning for NCI's clinical effort and recruitment of clinical staff. Besides Dr. Hertz, Mider had recruited Dr. Robert Smith as the surgical head for NCI, soon joined by Drs. Alfred Ketcham and John Potter. Dr. J. Robert Andrews was appointed head of Radiation Oncology and a little later was assisted by Drs. Philip Rubin and Herman Suit. In addition, Dr. Mider had assembled a group of internists into a General Medicine Branch. These physicians had a variety of interests in cancer and included, besides Steinfeld and White, Drs. John Fahey, James Holland, Stuart Sessoms, John Tuohy, Robert Sullivan, Donald Tschudy, Eugene Van Scott, and Donald Watkins. Most of them shared Mider's own interest in the metabolic effects of tumor growth. It was not until 1956 that Dr. Nathaniel Berlin, who came from the Donner Laboratory at Berkeley, CA, joined NCI and in succeeding years made the Metabolism Branch one of the most productive at the National Institutes of Health (NIH). Chemotherapy had Holland and Sullivan as protagonists. In 1955, Dr. David Rall transferred from Dr. Shear's laboratory and began the development of a remarkable pharmacology team.

In the care of cancer patients, the attending physicians were assisted by clinical associates. Because draft obligations could be satisfied by 2 years in the US Public Health Service, it was possible for the Institute to obtain many of the top graduates from American medical schools. This elite cadre of young internists and surgeons for the first time brought patients with disseminated cancer out of the dark recesses of nursing homes into the bright light of medical care of university quality. This happy circumstance not only provided a proper setting for the conduct of clinical investigation at NCI but also brought cancer research to the attention of a number of first-rate clinical scientists at the beginning of their careers. Many of the clinical associates later ascended the top rungs of the academic ladder and profoundly influenced the course of clinical investigation. The first associates at NCI were: *General Medicine*—Drs. Leonard Gold, Montague Lane, and Richard Silver in 1954 and Richard Fritz, Allan Levy, and William Mohler in 1955; and *Endocrinology*—Drs. James Pittman, Leslie De Groot, Herbert Levine, and Herbert Lubs.

The early studies in chemotherapy at NCI were conditioned by the lack of a drug development program and the resulting absence of candidate agents for clinical testing. Shear's program had ended just prior to opening of the Clinical Center, so that new drugs for study had to be obtained from drug companies or from friends in other programs. At that time, The Upjohn Co provided a riboflavin antagonist and uracil mustard. Holland, who had become interested in acute leukemia while at the Delafield Hospital, began studies of sequential corticosteroids, methotrexate, and the recently introduced mercaptopurine. Sullivan had started studies with perfusions of alkylating agents, and Hertz was exploring the effects of large doses of estrogen on postmenopausal breast cancer. Goldin was in the midst of a highly productive period and was publishing the results of studies in L1210 leukemia that became of great importance to the chemotherapy of clinical cancer in the 1960s. These included leucovorin rescue from antifolate toxicity, the importance of dose-response curves, the synergistic power of drug combinations, and the profound effects of intermittent dosage schedules of the antifolates in achieving maximum therapeutic benefit. In the design of these studies he had biostatistical help from Nathan Mantel and Samuel Greenhouse.

Dr. Mider became heavily involved in the expansion of laboratory research, and started looking for someone to take on the responsibilities for patient care and clinical investigation. Many of my former colleagues had come to the National Heart Institute and through them I met Dr. Mider. My background had been in the chemotherapy of infectious disease with emphasis on pharmacology and on design of clinical trials. I had been a junior participant during World War II in the highly successful drug development program for malaria devised by Drs. James Shannon and E. K. Marshall, Jr. After the war, I spent 7 years with Marshall at The Johns Hopkins University studying dosage schedules of penicillin and other antibiotics in mouse infections and applying the results to the treatment of pneumococcal pneumonia. A group of us from Baltimore had gone to St. Louis University in 1953, but things had not gone well and it was easy for Dr. Mider to convince me to become Clinical Director.

When I arrived in Bethesda in October of 1954, Holland had just accepted a post at Roswell Park Memorial Institute; we overlapped only a few months. In that period we agreed that it would make sense to join forces in the study of acute leukemia because of the scarcity of patients. We drew up a protocol that we could both live with, using a sequence of mercaptopurine daily with methotrexate every fourth day for induction, as compared to daily administration of both drugs given in sequence. This was the beginning (November 1954) of the first cooperative group, later to be named Leukemia B. The development of a clinical chemotherapy program had high priority, and more senior staff were needed. Dr. Emil Frei had been chief medical resident on our service at St. Louis University and had shown an interest in clinical investigation when we had undertaken a randomized trial of tetracycline and penicillin in pneumococcal pneumonia in the winter of 1953-1954. Frei joined me at NCI early in 1955 and Dr. Emil Freireich joined us a short time later. Freireich had become interested in hematology during a fellowship with Dr. Joseph Ross in Boston.

In 1955, most patients with acute leukemia died of sepsis and hemorrhage early in the course of the disease (4). Only a few patients lived long enough to observe the effects of antileukemic drugs. We began systematic studies of the causes of death in acute leukemia, and soon there were a number of studies relating granulocytopenia to various infections and the kinetics of thrombocytopenia to hemorrhage. To combat infections, intensive efforts were made to identify responsible organisms early and to treat them with massive combination antibiotics. These efforts paid off in terms of fewer deaths from infection, although at that time antistaphylococcal and antipseudomonas antibiotics were not effective. As to treatment of thrombocytopenia, platelet transfusions had not been accepted except on Dr. Sidney Farber's service, where Drs. Isaac Djerassi and Edmund Klein had been the pioneers. Classical hematologists had supposedly shown, using labeled (and therefore damaged) platelets, that the infused platelets did not last more than a few hours. In a brilliant series of studies, Freireich and Frei showed that most of the fatal hemorrhages occurred when the platelet count fell to $< 20{,}000/\text{mm}^3$ and that fresh whole blood (containing undamaged platelets) could raise the count to $> 20{,}000$ and prevent fatal hemorrhage (5). There were then a series of studies to harvest and preserve platelets. These studies were vigorously attacked by classical hematologists because they required larger amounts of blood than blood banks could easily obtain. I recall a dramatic showdown at a clinical staff meeting in 1956 where a motion was made to deny platelets to the NCI. In those days the entire clinical staff of the Clinical Center met monthly to discuss patient care problems. The other clinical directors came to our support and the motion was narrowly defeated. Later, of course, the NCI studies unequivocally demonstrated the great value of platelets in preventing hemorrhage, and platelet transfusions became one of the major elements in keeping leukemic patients alive until complete remission could be induced by drugs.

Development of a National Program in Chemotherapy (1,6)

Occasional complete remissions from alkylating agents given to patients with lymphomas, and from steroids, antifolate, and mercaptopurine given to patients with acute leukemia, led to deep interest on the part of Congress for a more systematic and planned program for the cure of cancer by chemotherapy. The scientific community was extremely resistant. It is said that scientists did not want a repetition of wartime programs for penicillin and malaria chemotherapy. In retrospect, this reasoning is hard to understand, inasmuch as these two efforts were spectacularly successful; however, their urgency and secrecy had left a bitter taste. In any event the resistance was real, and the National Advisory Cancer Council, the ultimate advisory body for NCI, opposed an engineered program. In December 1952, the Council told Congress that a chemotherapy program was unwarranted. Urged on by Mrs. Mary Lasker, Dr. Sidney Farber, and Dr. Cornelius Rhoads, Congress persisted and in 1953 appropriated 1 million dollars for leukemia research. NCI put the money into grants. It also responded by developing its intramural chemotherapy program and by seeking voluntary extramural cooperation through seminars and surveys. Congressional committees dourly observed these activities during 1954, concluded that these efforts did not constitute a chemotherapy program, and in 1955 directed NCI to start drug development. In May 1955 NCI formed the Cancer Chemotherapy National Service Center (CCNSC). The Center, however, was a compromise. Although NCI staff constituted a secretariat, intramural scientists were not included, and the decision-making powers were given to a large number of panels composed of extramural scientists and physicians. Dr. Kenneth Endicott was appointed chief of CCNSC and was successful in attracting several scientists such as Drs. Michael Shimkin, Robert Coghill (one of the stalwarts in wartime penicillin research), Leiter, and Hartwell to head divisions. Endicott quickly organized the secretariat and the various panels and had them spell out all the steps in a drug development program from synthesis through clinical trials. The panels attracted the best of scientists interested in chemotherapy, and they did a splendid job in specifying the needed elements. In brief, Endicott took these recommendations and used them to set up all the functions of a pharmaceutical house run by NCI but with the operation widely dispersed in industry and universities. To finance drug development, Endicott obtained permission to let contracts. Congress liked what it saw and appropriated large sums: fiscal year (FY) 1956 (beginning July 1, 1955), 5.15 million dollars; FY 1957, 20 million; and FY 1958, 25 million. At a later date, Endicott was succeeded by Dr. Sessoms, who was followed by Dr. Philip Waalkes.

While NIH policy forbade direct participation of intramural scientists in extramural activities such as the CCNSC, a few intramural scientists were elected to the panels and this provided some information exchange. Goldin, Law, and Marvin Schneiderman had important influences within the panels. In 1955, although I had no track record in cancer research, I was graciously invited to be a member of the Clinical Panel. At the first meeting of that panel, the late Dr. I. S. Ravdin, its chairman, needled me as a neophyte about clinical trials in cancer. I called attention to the clinical trials in infectious diseases, such as tuberculosis and pneumococcal pneumonia, and to those involving analgesic drugs, and I suggested that these principles be tried in cancer (7). The panel adopted this suggestion, and soon we were busy designing a model trial to examine feasibility. To write a protocol and carry out the trial, a group was formed which represented several institutions: Roswell Park—Holland, Oleg Selawry, and William Regelson; Johns Hopkins—Louis Lasagna and Albert Owens; Lemuel Shattuck Hospital—Thomas Chalmers; NCI—Freireich, Frei, Carbone, and Zubrod; Georgetown—Bruce Shnider; and University of Miami—Ralph Jones and Jacob Colsky. These investigators quickly formed the Eastern Cooperative Group and started a comparison of mechlorethamine and thiotepa in breast cancer, Hodgkin's disease, and melanoma. It was in these sessions that the classic principles of clinical trials were applied to the study of cancer chemotherapy. The Clinical Panel kept a careful eye on this trial and decided to encourage the formation of other groups based on this model, so that known drugs could be calibrated in a variety of cancers. The panel formed an experimental design subcommittee, whose members were Wayne Rundles (hematology); Arnold Seligman (surgery); Milton Wittenborg (radiation); Schneiderman (biostatistics); and Zubrod (pharmacology). Later members were George Escher, Johannes Ipsen, and Lasagna. I chaired the group, and over a period of 3 years, the subcommittee reviewed every protocol coming from the growing number of cooperative groups. This mechanism rapidly schooled a large number of oncologists in clinical trial design and operation and the need to have biostatisticians and pharmacologists participate in the early stages of clinical trials.

Funding the cooperative groups was a problem. The development of an integrated chemotherapy could not be carried out through the traditional grants mechanism. At that time as a highly sheltered intramural scientist, I was unaware of the events taking place in the Division of Research Grants. Dr. Palmer Saunders wrote to me about this history and has kindly given me permission to extract the following:

"The purpose of the extramural programs of the National Institutes of Health according to the principles laid down by Vannevar Bush was to supervise the award of funds to universities to conduct research in the public interest. Dr. Bush mandated that the supervision and control of these funds should be in the hands of a group 'which does not conduct research of its own'; hence, the objective of the very careful efforts initiated by Dyer and Sebrell and continued by Shannon was to create a fairly rigid wall between the administration of the extramural programs and that of the intramural research activities of NIH.

"The creation of the CCNSC was a breach of Dr. Bush's principle in that it permitted the National Cancer Institute to award research funds under a contract program administered by an intramural scientist, although, as you commented, there was a division between the actual intramural scientists in the laboratories and the administrators of the program itself (Endicott, Leiter, et al). In my opinion it was this rigid insistence on a separation of study sections from all program input that led to Dr. Endicott's concern with study sections and his eventual retention of the special virus leukemia program entirely within the contract-intramural mechanism. The conflict between the CCNSC itself and the study section review mechanism was exacerbated by the creation of the chemotherapy study section in the Division of Research Grants and the assignment to it of the responsibility for the review of clinical cooperative group research protocols. This immediately created a double review situation since the CCNSC had already undertaken to provide funds to various clinical cooperative groups and had itself approved certain protocols. When these protocols were incorporated into regular research grant applications and reviewed by the cancer chemotherapy study section the result often was a rejection of a previously approved protocol. (When the cancer chemotherapy study section was formed out of the old pharmacology and experimental therapeutic study section, the other part of the study section was renamed the pharmacology study section and I was brought in from the Chemical Corps Medical Laboratories to become its executive secretary.) Dr. Endicott appealed to Dr. Ernest Allen, then Director of the Division of Research Grants, to obtain an executive secretary for the study section, an individual who might be more sympathetic to the needs of the CCNSC. Dr. Allen brought me from the Allergy and Infectious Diseases Institute to take over the cancer chemotherapy study section. It became apparent that no real review of the clinical cooperative trials within the format of the study section was possible. We recommended to Dr. Sessoms (then CCNSC Director) that the center should take over the review of the clinical cooperative trials program and that the study section should be relieved of all responsibility for the review of organized protocol programs. This recommendation was accepted and from that time on the chemotherapy study section was no longer involved in the review of organized clinical cooperative group grant proposals."

First Cure of Metastatic Cancer—an NCI Operation

In 1955, while the clinical services at NCI were building and the CCNSC was getting underway, Dr. Roy Hertz's Endocrinology Branch was in full swing. Transferred intact from George Washington Medical School, the staff was increased by Hertz. Dr. Delbert Bergenstal, an associate of Dr. Charles Huggins in his endocrinology research, came from the University of Chicago. Dr. Min Chiu Li, a Canton-born physician just finishing a fellowship, came from Memorial Sloan-Kettering. While in New York working with Dr. Olaf Pearson, he had noted a fall in urinary gonadotropin titer in a patient with melanoma after the administration of methotrexate (8). In 1944, Hertz (9) had shown that antifolates interfered with estrogen effect on the chick oviduct. Li and Pearson tested this hypothesis in a girl with melanoma. Estrogen effect was not abolished by the methotrexate, but coincidentally the gonadotropins, presumably made by the melanoma, fell to normal levels. At that point, Li moved to NCI, but before leaving he mentioned to Dr. Mortimer Lipsett, another colleague of Pearson's, that he intended to try methotrexate in choriocarcinoma. Li convinced Hertz that this trial should be undertaken: a generous concession, because it involved a considerable diversion of Hertz's laboratory team from other major projects. At that time Condit in the Medicine Branch was studying the pharmacology of single large doses of methotrexate in patients. He had been intrigued by the increased therapeutic efficacy in L1210 leukemia of intermittent methotrexate demonstrated by Goldin in 1954 and was exploring the safety of intermittent schedules in patients. Condit, Li, and Hertz started with a single dose to a patient with metastatic choriocarcinoma on October 20, 1955, hoping gradually to develop an intermittent regimen. When the urinary gonadotropin promptly fell, the decision was made to go beyond the existing pharmacologic data, and this led to a 4-5-day course, administered repeatedly after recovery from the considerable toxicity. The fall in gonadotropins encouraged continued chemotherapy. By December the pulmonary metastases were smaller and by early February 1956 all evidence of the disease had disappeared. A second patient in March, and a third in April, repeated this experience. Li, Hertz, and Spencer published a cautious note in October 1956 (10), but as more and more patients flocked to NCI for the rumored cure, further trials demonstrated that intermittent methotrexate did indeed cure one type of metastatic cancer (11). Hertz recruited Drs. Mortimer Lipsett, William Odell, and Griff Ross, and, together with clinical associates Drs. John L. Lewis, Richard Moy, Leo Stolbach, Charles Nadler, David Goodman, Theodore Goodfriend, and Saul Genuth, they constituted an extraordinary team of clinical investigators. Hertz and his colleagues published a large number of important papers on trophoblastic tumors, as well as a number concerned with the discovery of the effects of OP'DDD in adrenal tumors. Del Bergenstal died at age 42, in 1959. Li returned to Memorial Sloan-Kettering, left for a post at Nassau Hospital, and died in 1980. Dr. Hertz retired in 1965, and by 1966 the team had begun to drift apart. By 1970 all of the early participants in the choriocarcinoma research had left NCI.

Except for one, the elements that had made the first cure of metastatic cancer possible came from NCI scientists: Hertz's leadership of the Endrocrinology Branch and its clinical and laboratory teams, Law's L1210 leukemia, Goldin's observations on intermittent methotrexate, Condit's pharmacologic studies, and Hertz and Spencer's precise gonadotropin assays. Dr. Li, an NCI scientist from 1955 to 1957, brought from his one patient the idea of relating methotrexate to choriocarcinoma and provided the spark that ignited the fire. But this idea, too, came from Hertz's fine fundamental research in the 1940s on antifolates and their interference with the estrogen effect on the chick oviduct.

NCI's Contribution to the Cure of Acute Lymphocytic Leukemia (12,13)

In addition to their studies of infection and hemorrhage as causes of early death in acute lymphocytic leukemia (ALL) of childhood, Frei and Freireich were deep in studies of drug effects. Until 1958, as internists, they had had to care for all of the children as well as adults, but in 1958 we were at last able to recruit a pediatrician, Dr. Myron Karon. Gerald Bodey, Evan Hersh, and Boyd Nies had come aboard as clinical associates and remained on the staff. Drs. Paul Carbone, Bruce Shnider, Oleg

Selawry, and Clyde Brindley came on in more senior roles. Edmund Gehan joined the group to give biostatistical input. Frei had become chairman of the Leukemia B group, which by 1959 had grown to > 12 members. Pilot drug studies, carried out at NCI and at Roswell Park by Holland, were quickly tried on a larger scale by Leukemia B. The emphasis was on using methotrexate, mercaptopurine, and prednisone to improve the percentages of complete remission of ALL. The controlled clinical trials sponsored by the group had been considerably refined and had shown the importance of distinguishing between remission induction and remission maintenance; the most important indices of drug effectiveness were percent of induced complete remissions and their duration. The group had also added cyclophosphamide as a fourth drug producing complete remissions. Vincristine had been made available in 1960 by Eli Lilly to four or five investigators. Karon, Freireich, and Frei used it in somewhat larger doses and were first to establish it as an important remission inducer.

In 1960 Dr. Endicott became Director of NCI; in the following year, I was made the Scientific Director and Dr. Berlin, Clinical Director. The three of us enjoyed close working relationships in strengthening NCI programs; Berlin and I shared common objectives for excellence in intramural research and for the next dozen years used our resources toward that goal. With the many opportunities to study new concepts and new drugs, NCI expanded its clinical resource not only at the Clinical Center but also in satellite hospitals. Relations with the Baltimore Public Health Service Hospital, which had continued since the early 1930s, became more extensive when NCI supported the conversion of the nurses' residence into a laboratory building. In addition, a discrete clinical service for oncology was established. Drs. Arthur Serpick and Jerome Block moved from Bethesda to this unit and were joined by Drs. Nicholas Bachur, Carl Levy, and Viola Horvath. A clinical associate program was developed and from this came Drs. Steven Schimpff, Charles Schiffer, Peter Wiernik, Richard Kaplan, and John Ruckdeschel. Block ran the unit until 1971 when he became Deputy Director of the Clinical Center and was succeeded by Dr. Michael Walker. At a later date, because of the lack of research in lung cancer at NCI, the Veterans Administration Hospital in Washington, DC, agreed to the establishment of a clinical and laboratory unit. Dr. Oleg Selawry headed this effort with Drs. Patrick Creaven, Martin Cohen, Francisco Tejada, and Lawrence Broder.

Soon after the reorganization in 1961, I proposed to Dr. Endicott that with five drugs active in acute leukemia and a growing ability to control infection and hemorrhage, we should form an NCI task force to engineer the cure of acute leukemia. He liked the idea but thought we should learn more about task forces, so we visited friends at IBM where such groups had been used with great effectiveness. The key elements in their success seemed to be selecting a single objective; identifying the best people in the company for the job; giving them the time and resources to get it done; and disbanding the task force as soon as the objective was within reach. I obtained backing for the idea of a task force from Drs. Ravdin, Farber, and Sessoms, whose support was essential in matters relating to the CCNSC. On May 7, 1962, we held the first meeting of the Acute Leukemia Task Force. My purpose was to bring together at frequent intervals laboratory scientists, clinical investigators, statisticians, pharmacologists, and especially the staff and contractors of the CCNSC. CCNSC had grown into a highly efficient drug development operation under Endicott, but exchange with intramural scientists was still episodic. The composition of the task force varied, depending on the topic under discussion, but always included scientists from CCNSC, NCI clinical investigators and basic scientists, contractors such as Howard Skipper and Frank Schabel from Southern Research Institute and Charles Kensler of A. D. Little Co, members of Leukemia Groups A and B, and physicians from independent institutions including Roswell Park, Memorial Sloan-Kettering, and St. Jude. There was great enthusiasm by the members of the task force, and during its first year, monthly meetings were held.

The first approach of the task force was to review all data relative to ALL, whether the source was CCNSC and its contractors, the intramural operation, Leukemia Groups A and B, or individual scientists and their grants. Small subgroups made intensive studies of the predictability value of the various screens then in use, and for the first time it became clear that L1210 was far superior to other rodent tumors in selecting drugs for ALL. Another group reviewed the data on toxicity of antileukemia drugs in various species and interspecies correlations. This led to the demonstration that, quantitatively, drugs were equitoxic for most species, provided that the conditions of trial were identical and that body surface rather than weight was used to calculate dosage (14). The group also reviewed qualitative toxicity prediction (that is, the value of various animal species in predicting specific organ toxicity for patients) and found reasonably good correlations, although false-positive and false-negative predictions occurred (15). This review of existing data quickly brought a good deal of insight into the safety of the early phases of new drug introduction to patients and led to the decision to use L1210 to model the cure of childhood ALL. Other subgroups tackled the problems of infections and hemorrhage, and contracts were soon established to study the collection and preservation of platelets and granulocytes. At the same time, Freireich had begun working with George Judson of IBM (whose son had leukemia) on the first blood cell separator, and reports of their success provided exciting news at each meeting. So too did the NCI efforts in developing "Life Islands" and laminar air flow rooms for protection of the granulocyte-depleted leukemic patients. This research had been started by Frei and Dr. Stanley Schwartz and was continued by Seymour Perry after Frei left NCI. One of the rising stars in the NCI group was Robert Levin, who died in an airplane accident on his way to an experimental hematology meeting in Oak Ridge. Joseph Burchenal, head of Leukemia Group A which he had organized in December 1955, was fully cognizant of all the research at Memorial Sloan-Kettering and was a tower of strength at these meetings. He not only was an expert in the response of various animal leukemias to drugs but also provided exciting clinical findings that kept morale high. Burchenal had started his study on long-term survivors of acute leukemia by asking members of the American Society of Hematology as well as friends abroad to document all patients with acute leukemia who had survived $\geqslant 5$ years. He found 103 such patients, still living and well, all treated with drugs; more importantly, he could find no long-term survivors who had not received chemotherapy (16). While these patients represented $< 1\%$ of all acute leukemias, it was encouraging to postulate that drugs occasionally "cured." Burchenal also brought reports of his trips to Uganda to meet Denis Burkitt and of the early successes in complete remission of Burkitt's lymphoma following dactinomycin and cyclophosphamide (17). Skipper and his colleagues at Southern Research Institute were in the midst of their superb work using L1210 in highly quantitative fashion to analyze the best chemotherapy for leukemia. In 1962, Mortimer Mendelsohn (18) published his classic work on growth fractions, suggesting that tumors with high proportions of dividing cells were exquisitely sensitive to antitumor drugs. Skipper immediately recognized the importance of these observations and undertook further study in his quantitative L1210 model. At the monthly meetings Skipper emphasized that to kill the last leukemic cell, one had to continue intensive treatment long after apparent complete remission had been induced (19). This was taken up by the clinicians in their design of clinical trials. Drug combinations which had always been avoided because of severe drug toxicity were beginning to be studied under the protective umbrella of "prophylactic" platelet replacement. In 1959, Li, now back at Memorial, had shown the remarkable effects and safety of a three-drug combination in a few patients with testicular cancer (20). At about the same time the Leukemia Group B had undertaken a comparative study of combinations of mercaptopurine and methotrexate in ALL. Published in 1961, the data made clear that two-drug combinations were better remission inducers than either drug alone (13). In a pilot study at NCI, Karon, Freireich, and Frei had shown the remarkable effects of vincristine and prednisone in combination, and this was confirmed in a Leukemia Group B study. Combination chemotherapy was clearly the right direction, both for remission induction and remission maintenance, and the key question was how best to use the five drugs active in ALL. Frei and Freireich (13) had calculated, with lots of assumptions, that a single leukemic cell would proliferate to clinically evident leukemia in about 165 days. Therefore, it was argued that a complete remission lasting > 165 days after treatment was stopped must approximate killing of the last surviving leukemic cell. Freireich, Karon, and Frei, after prolonged discussion at the Task Force, designed the VAMP regimen (vincristine, methotrexate, mercaptopurine, and prednisone) to test this hypothesis. In 13 patients receiving VAMP at NCI, the median duration of remission was 154 days and three patients went well beyond the 165th day (13). The two leukemia groups and Donald Pinkel (now transplanted from Roswell Park to St. Jude) immediately developed studies based on these principles, and those studies eventually led to the proof of cure in ALL. Relapses in the central nervous system held down the cure rate until the Leukemia Group B used "prophylactic" intrathecal methotrexate and the group at St. Jude had combined this with x-irradiation of the brain and spinal cord, successfully preventing relapses.

The success of the Task Force and of combination chemotherapy had immediate scientific and political repercussions. On the scientific side, it was apparent that other drug-sensitive cancers should be studied under the same strategies aimed at cure. Advanced Hodgkin's disease was 100% fatal in 1963 and was generally treated with single agents because of the fear of toxicity of combinations.

However, Hodgkin's disease was the next best target because not only was it known that alkylating agents and corticosteroids were active, but Frei and Carbone and others in the Eastern Cooperative Study Group had shown that the vinca alkaloids and methotrexate were also active in lymphomas as single agents, although vinblastine was shown to be superior to vincristine in their study (21,22) In 1965, Mortimer Lacher and John Durant (23) at Memorial published data showing that vinblastine and chlorambucil were active in combination. At NCI, attention turned to four-drug combinations. In 1963, Drs. Vincent DeVita, John Moxley, and Emil Frei developed the first four-drug combination for patients with Hodgkin's disease.

The first combination, MOMP (cyclophosphamide, vincristine, methotrexate, and prednisone), was used together with irradiation. The first patient was treated at NCI on September 27, 1963, by DeVita and Moxley. In 1965, DeVita gave a paper at a meeting of the American Association for Cancer Research on the results of treatment in the first 14 patients (24). While MOMP was highly effective, procarbazine had recently been shown to have activity at NCI and in France and was substituted for methotrexate. The resulting MOPP combination was given to the first patient at NCI on August 5, 1964, and in early 1965 several more patients were added. Data on the first 43 patients demonstrated that MOPP had quadrupled the complete remission rate to 80% and prolonged relapse-free survival (25,26). The Medicine Branch beautifully followed up the MOPP study and organized combination chemotherapy studies in the non-Hodgkin's lymphomas that proved these diseases, too, could often be cured by combination chemotherapy (27-29).

Politically, the successes in acute leukemia led to a demand mostly from members of the National Advisory Cancer Council and from Congress that task forces be formed for virtually every type of cancer. Frei chaired a Lymphoma Task Force, which in its 3-year existence brought together radiation therapists, chemotherapists, and hematologists. There were task forces formed for clinical trials, solid tumors, chronic myelocytic leukemia, and brain tumors. NCI through intramural resources formed the Breast Cancer Task Force, the Lung Cancer Working Party, the Brain Tumor Study Group, and the Polycythemia Vera Study Group. Drs. Joseph Glass, Joseph Newman, and Stephen Carter successively helped with the operations of these groups. An offshoot was the formation of the highly successful National Adjuvant Study Breast Cancer Program, under Dr. Bernard Fisher. He pointed out that university centers rarely saw large numbers of early breast cancer patients, who usually were cared for in community hospitals. He organized community physicians in an adjuvant study using melphalan. Carbone played a major role in designing the study. Carbone also took the initiative in interesting Dr. Gianni Bonadonna in Milan to undertake a contract with NCI to support disease-oriented clinical research under contract. As part of this effort, Carter formed the Gastrointestinal Study Group.

The Acute Leukemia Task Force continued meeting through 1966, but its objective of coordination of research efforts and cure of a single disease entity had been accomplished, and, in keeping with the original concept, it was officially disbanded in 1968. It had shown that given several highly active drugs in a single disease plus a body of knowledge on its natural history, collaboration of all those who had the data and resources could speed up the cure of that disease. The Task Force had also shown the need to break down communication walls to provide a mechanism for analysis and rapid exploitation of information coming from widely scattered sources—grantees, contractors, intramural research, and cooperative groups. During the hot pursuit of the cure of childhood ALL and when we did not yet appreciate what its lessons meant, an outside event occurred that, despite all the turmoil it created at the time, proved to be a great blessing for chemotherapy research at NCI.

The Richardson Report

In February 1965, while choriocarcinoma and ALL were being cured, the Wooldridge Committee, an outside group studying the NIH, made a report which included these comments: "... the operations of CCNSC have been less distinguished scientifically than the traditional research programs supported by the NIH ..., and we recommend ... a throughgoing review of the administration and management of collaborative programs. ..."

The senior intramural staff of NCI, not part of the CCNSC except in some of the indirect ways already mentioned, felt that the Wooldridge Report had missed its mark because NCI had never intended that the CCNSC conduct research but rather that it provide service and materials that would help the scientists and physicians of the country in their research. Had the members of the committee interviewed either intramural or CCNSC staff, the Committee might have understood NCI's objectives for the CCNSC. But this they did not do, and most of us concluded the Wooldridge Report was a symptom of relapse of the chronic conviction of the scientific community that the engineered approach to biomedicine is necessarily bad.

As Scientific Director of NCI, I was asked by Shannon and Endicott to head an NCI group to review CCNSC and, if required, to suggest improvements. The working group included Drs. Saul Schepartz, Carl Baker (later to succeed Endicott as Director), and Mr. Louis Carrese, who was a systems analyst and planner from Dr. Endicott's office. Many of the NCI staff served as consultants, especially Endicott, Rall, Venditti, Frei, and Leiter. We met almost daily for 4 months, looking at all the data from CCNSC, the cooperative groups, and the intramural area. Our job was made easy because the Acute Leukemia Task Force had already done much of the work, and we used these lessons to fashion a new chemotherapy program based on linear array decision-making concepts. We chose as the scientific base of the program the cell kinetic relationship to drug sensitivity and the rapidly evolving knowledge about pharmacologic disposition of antitumor drugs. In July, we presented the results of our labors to Dr. Shannon and in August to the NCAB. Both approved, and I was asked to head a new chemotherapy effort that combined CCNSC with the intramural activities relating to drugs. Dr. Shannon also asked a group of extramural consultants to examine the new NCI plans for chemotherapy. The committee was chaired by Dr. Arthur Richardson and had as members Drs. Louis Goodman, A. McGehee Harvey, Thomas Chalmers, Arthur Cope, Oscar Creech, Jr, Harry Dowling, Franklin Ebaugh, Jr, Thomas Maren, Richard Mason, John Taggart, and Paul Calabresi. Dr. J. Palmer Saunders came from the Division of Research Grants to be the Deputy Director of our new chemotherapy program and Executive Secretary to the Richardson Committee. After a prolonged study, a report was issued which gave approval to past efforts and to the new plan and made a number of recommendations. The report was never circulated, possibly because there was no desire to confront those segments of the scientific community who were irretrievably biased against the CCNSC. One of the committee's observations was the enormous impact that organized clinical trials had had upon upgrading care of cancer patients nationally. The cooperative groups were assigned to the program. Dr. Saunders formed the CCIRC and brought in Dr. Lawrence Foye to get it started.

The New Chemotherapy Program (1)

The chemotherapy program began to function in the new mode in 1965. This year also marked the move of Frei to M. D. Anderson; he took with him a number of NCI staff—Freireich, Karon, Hersh, Bodey, Gehan, Ti Li Loo, and later, Robert Livingston and Jeffrey Gottleib. Because of the early deaths of Myron Karon and Jeff Gottleib, cancer research lost two of its most productive clinical investigators.

The new chemotherapy effort, with its admixture of intramural and CCNSC components, slowly produced new leadership. Dr. Seymour Perry became Deputy Director when Dr. Saunders was made Chief of Research Grants. The other senior leaders were: *Experimental Therapeutics*—Rall; *Drug Development*—Schepartz; *Medical Oncology*—Carbone; *Cancer Therapy Evaluation*—Carter; and *Baltimore Cancer Research Center* (BCRC)—Jerome Block, later succeeded by Walker. In 1965, the program established a decision network group that contained all the responsible lab chiefs and section heads. The network met every 2 weeks and reviewed every active drug to decide if it was ready to move ahead. The first year was spent in devising new criteria for decision-making in screening, toxicology, pharmacology, and clinical trials. Once established, these criteria were applied to each compound that showed activity. We tried to extend Endicott's concept of a pharmaceutical house within the government, which would move drugs rapidly and safely into clinical trial. Unlike a pharmaceutical house, however, our corporate interest was not merely in those compounds that arose uniquely from NCI screens. We had interest in each compound from around the world that showed promise either experimentally or clinically. If it passed the NCI criteria, every effort was made to develop the additional data necessary to obtain Investigational New Drug (IND) approval and move the compound into broad clinical studies. In this way, drugs such as cytarabine, doxorubicin, and later bleomycin and cisplatin, were introduced into widespread trial in the United States. The primary screen became a necessary instrument to provide data on activity so that decisions could be made concerning which drugs to move toward work-up and clinical trial. On its own, the screen turned up only the nitrosoureas, dacarbazine, and mithramycin.

The new chemotherapy program, though founded on the operational principles developed in the Acute Leukemia Task Force, no longer had this group to turn to, as it had been inactive after 1966. In its place, I formed the Atlanta Group which met in that city about six times a year. Composed of NCI senior staff, contractors Howard Skipper and Charles Kensler, and a large number of clinical investigators from NCI, Memorial Sloan-Kettering, Roswell Park, and M. D. Anderson, the group continued to review all the data coming from NCI's drug development program. The drugs of greatest interest at that time were nitrosoureas, cytarabine, thioguanine, and doxorubicin. These discussions influenced combination trials in acute myeloblastic leukemia, which at that time was always fatal. There were also heroic attempts to use the measurements coming from cell kinetics to achieve curative regimens for the small-growth-fraction tumors—the carcinomas. But with the failures over the years, this hope gradually faded.

The intramural clinical operation, depleted by the loss of the Frei-Freireich team, had taken on in addition to its patient care and clinical investigational responsibilities the clinical duties relating to the national drug research program. With the extraordinary talents of Carbone, the rebuilding of the clinical team began. Fortunately, the clinical associate program together with outside recruitments had provided a group of superb clinical investigators who began to fill the gap. Edward Henderson headed the leukemia studies. Carbone was joined by DeVita, Moxley, and George Canellos in 1963. DeVita later succeeded Carbone as Chief of the Medicine Branch. The clinical and research associates of 1965 included Robert Gallo; in 1966, John Ziegler, Charles Vogel, and Herbert Kahn; in 1967, Robert Young, Arthur Levine, Robert Graw, Bruce Chabner, and Charles Haskell. Recruited from outside were Brigid Leventhal, Jacqueline Whang Peng, and Richard Simon. The major activities clinically were: rapid pursuit of cures of ALL, lymphomas, and childhood cancers; chemotherapy of advanced breast cancer; further development of the IBM cell separator; improvements in the "Life Islands" and laminar air flow rooms; granulocyte transfusions; studies of the natural history of chronic myelocytic leukemia; and cell kinetic research.

Dr. David Rall as head of experimental therapeutics had built up an extraordinary team of pharmacologists. These included Kurt Kohn, Jack Davidson, Ti Li Loo, David Johns, Vincent Oliverio, Richard Adamson, Bruce Chabner, Philip Schein, Joseph Fenstermacher, Victor Levin, Archie Bleyer, Ronald Blasberg, Daniel Zaharko, Vincent Bono, David Cooney, and Anthony Guarino. They were into every aspect of research on the pharmacologic disposition of antitumor drugs and were particularly productive in the study of the fundamental aspects of blood-brain barrier phenomena. Zaharko, with chemical engineers Bishoff from the University of Maryland and Robert Dedrick from the NIH, was instrumental in developing the modern concepts of pharmacokinetics.

Rall and his group were highly successful in developing the basic principles of toxicology and pharmacology for the new drug development network. Rall was made Director of the National Institute of Environmental Health Sciences in 1971 and was succeeded by Oliverio.

At a later date the program added on the Uganda Cancer Center, as a result of the efforts of Carbone. When it was learned that Denis Burkitt was leaving Uganda, in 1966, Burchenal suggested that NCI fill the gap. Carbone visited Uganda and proposed a Lymphoma Treatment Center to be established at Makerere University, but geographically separate from the huge Mulago Hospital. We decided to support the center, and in 1967, Dr. John Ziegler, a clinical associate, went to Uganda. He organized his elegant trials for the study of Burkitt's and Hodgkin's lymphomas. Sir Ian McAdam and Drs. Sebastian Kyalwazi and Charles Olweny, all of Makerere, were sources of strong support in the clinical research that developed. Ziegler was soon followed by Drs. Vogel, Aron Primack, Avrom Bluming, and later Ian Magrath. The effort was expanded to a second unit, the Solid Tumor Center, which opened in 1969. Here under Vogel there were pioneering studies of melanoma in native Ugandans, hepatoma and Kaposi's sarcoma. The Uganda Cancer Institute was extraordinarily productive, thoroughly established the role of drugs in the cure of Burkitt's lymphoma, called attention to the possible role of hepatitis in hepatoma, and did the classic studies in the chemotherapy of Kaposi's sarcoma. It also served as a focal point for innumerable scientists interested in Burkitt's lymphoma as a possible viral disease, in its immunologic features, and in additional epidemiologic studies. As the political situation in Uganda deteriorated, the NCI staff left the country, one by one, until all were gone by 1973. Charles Olweny, who had spent 2 years in Bethesda, became the director, and the center continued vigorously under his leadership.

The Division of Cancer Treatment

In 1971, Congress, urged on by Mary Lasker, Sidney Farber, Lee Clark, and Solomon Garb, passed the National Cancer Act in a bipartisan collaboration. The successes in the cures of choriocarcinoma, Burkitt's lymphoma, ALL, and Hodgkin's disease, as well as the emerging data on tumor viruses, were powerful influences in the success of this legislation. President Nixon became a strong advocate and signed the Act on December 23, 1971, with considerable ceremony and surrounded by all the beauty of the White House at Christmas. The Act not only infused 100 million dollars into cancer research, it created the President's Cancer Panel with its bypass budget, enormously expanded the Cancer Centers program, and led to the development of the National Cancer Program. NCI became a bureau, and its major programs became Divisions. Cancer research had moved irretrievably into the political arena, and this change had many impacts, beyond the scope of this memoir, upon NCI and chemotherapy research.

The major objective of the Chemotherapy Program formed in 1966 was research in the cure of cancer by chemotherapy. The successes against the cancers of the young—the large-growth-fraction tumors—by combining curative drugs with surgery and ionizing irradiation led to a call to apply multimodality treatment to all tumors. This resulted in the transformation of the Program into a Division of Cancer Treatment. Perhaps this may have been premature, for the small-growth-fraction tumors, ie, the common carcinomas, had not yet (and still have not) been provided with curative drugs. In hindsight, one might say that the single-minded purpose of a division of research chemotherapy might have been more fruitful than one of treatment, distracted by the attempts to cure the common carcinomas by multimodal therapy at a time when truly active drugs were still wanting. In any event, this move away from an undistracted single goal provides a natural ending for this personal account of chemotherapy research at NCI.

REFERENCES

1. ZUBROD CG, SCHEPARTZ S, LEITER J, ET AL. The chemotherapy program of the National Cancer Institute: history, analysis, and plans. Cancer Chemother Rep 50:349-540, 1966.
2. SHIMKIN MB. As memory serves—an informal history of the National Cancer Institute, 1937-57. JNCI 59:559-600, 1977.
3. ZUBROD CG. Selective toxicity of anticancer drugs: Presidential Address. Cancer Res 38:4377-4384, 1978.
4. HERSH FM, BODEY GP, NIES BA, ET AL. Causes of death in acute leukemia. A ten year study of 414 patients from 1954-63. JAMA 193:105-109, 1965.
5. FREIREICH EJ, KLIMAN A, GAYDOS LA, ET AL. Response to repeated blood transfusions from the same donor. Ann Intern Med 59:277-287, 1963.
6. ZUBROD CG. The Drug Development Program of the National Cancer Institute: its history, results and impact on marketing. *In* Orphan Drugs. New York and Basel, Marcel Dekker, 1982. Drugs and the Pharmaceutical Sciences, vol 13, pp 141-152.
7. ZUBROD CG. Clinical trials in cancer patients: an introduction. Controlled Clin Trials 3:185-187, 1982.
8. LI MC. Trophoblastic disease: natural history, diagnosis and treatment. Ann Intern Med 74:102-112, 1971.
9. HERTZ R. Interference with estrogen induced tissue growth in the chick genital tract by a folic acid antagonist. Science 107:300, 1948.
10. LI MC, HERTZ R, and SPENCER DB. Effect of methotrexate upon choriocarcinoma and chorioadenoma. Proc Soc Exp Biol Med 93:361-366, 1956.
11. HERTZ R, LEWIS J, and LIPSETT MB. Five years experience with the chemotherapy of metastatic trophoblastic diseases in women. Am J Obstet Gynecol 86:808-814, 1963.
12. ZUBROD CG. Historic milestones in curative chemotherapy. Semin Oncol 6:490-505, 1979.
13. FREI E, III, and FREIREICH EJ. Progress and perspectives in the chemotherapy of acute leukemia. Advances Chemother 2:269-298, 1965.
14. FREIREICH EJ, GEHAN EA, RALL DP, ET AL. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50:219-244, 1966.
15. SCHEIN PS, DAVIS RD, CARTER S, ET AL. The evaluation of anti-

cancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man. Clin Pharmacol Ther 11:3-40, 1970.

16. BURCHENAL JH. Long term remissions in acute leukemia. *In* Chemotherapy of Cancer (Plattner PA, ed). Amsterdam, Elsevier, 1964, pp 257-262.
17. OETTGEN HF, CLIFFORD P, and BURKITT D. Malignant lymphoma involving the jaw in African children: treatment with alkylating agents and actinomycin D. Cancer Chemother Rep 28:25-34, 1963.
18. MENDELSOHN ML. Autoradiographic analysis of cell proliferation in spontaneous breast cancer of C_3H mouse. III. The growth fraction. JNCI 28:1015-1029, 1962.
19. SKIPPER HE, SCHABEL FM, JR, and WILCOX WS. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemia. Cancer Chemother Rep 35:3-111, 1964.
20. LI MC, WHITMORE WF, JR, and GOLBEY R. Combined drug therapy upon metastatic choriocarcinoma. Proc Am Assoc Cancer Res 3:37, 1959.
21. FREI E, III, SPURR CL, BRINDLEY CO, ET AL. Clinical studies of dichloromethotrexate (NSC-29630). Clin Pharmacol Ther 6:160, 1965.
22. CARBONE PP, SPURR C, SCHNEIDERMAN M, ET AL. Management of patients with malignant lymphoma: a comparative study with cyclophosphamide and vinca alkaloids. Cancer Res 28:811-822, 1968.
23. LACHER MJ, and DURANT JR. Combined vinblastine and chlorambucil therapy of Hodgkin's disease. Ann Intern Med 62:468-476, 1965.
24. DEVITA V, MOXLEY JH, III, BRACE K, ET AL. Intensive combination chemotherapy and X-irradiation in the treatment of Hodgkin's disease. Proc Am Assoc Cancer Res 6:15, 1965.
25. DEVITA VT, and SERPICK A. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Proc Am Assoc Cancer Res 8:13, 1967.
26. DEVITA V, SERPICK A, and CARBONE PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 73:881-895, 1970.
27. DEVITA VT, SIMON RM, HUBBARD SM, ET AL. Curability of advanced Hodgkin's disease with chemotherapy: long-term follow-up of MOPP treated patients at NCI. Ann Intern Med 92:587-595, 1980.
28. SCHEIN PS, CHABNER BA, CANELLOS GP, ET AL. Potential for prolonged disease-free survival following combination chemotherapy of non-Hodgkin's lymphoma. Blood 43:181-189, 1974.
29. DEVITA VT, CANELLOS GP, CHABNER B, ET AL. Advanced diffuse histiocytic lymphoma, a potentially curable disease. Results with combination chemotherapy. Lancet 1:248-250, 1975.

Intramural Therapeutic Research at the National Cancer Institute, Department of Medicine: 1955-1965

Emil Frei, III[1,*]

One year after the opening of the Clinical Center of the National Institutes of Health (NIH) in 1953, Dr. Gordon Zubrod was appointed Clinical Director of the National Cancer Institute (NCI) and assumed broad responsibilities for the development of experimental and clinical programs relating to cancer chemotherapy. In contrast to the general beliefs of the broader scientific community, Dr. Zubrod had an abiding faith that major progress could be achieved in the systemic treatment of cancer by the integrated application of basic and clinical science. His inspiration, administrative support, and direct scientific input made possible what followed.

I arrived at NCI in early 1955, and Dr. Emil J Freireich joined me some 6 months later. With Drs. Zubrod and James F. Holland, who had initiated the NCI clinical leukemia program, we decided that a major initiative for the Medicine Branch would relate to therapeutic research in acute leukemia. This decision was made for a variety of reasons, including the following: therapeutic leads in terms of the activity of folic acid and purine antagonists and of corticosteroids had already been developed; tumor could be sampled and studied from the peripheral blood and particularly from the bone marrow; and several basic scientists with whom we collaborated, particularly Drs. Lloyd Law and Abraham Goldin, had major experimental programs relating to leukemia, including its therapy.

The setting for this major new initiative in therapeutic research in cancer also deserves description. In 1955, the Clinical Center was a large, new facility where some one-third to one-half of the beds were empty and one-third of the laboratories were not as yet occupied; many of the other laboratories were only partially occupied. For the young investigator, there was a minimum of administrative overlay. Patient care income was not a determinant of one's success or salary; resources, in terms of patients, laboratory space, equipment and supplies, for example, were not rate-limiting. Indeed, research was the only goal at NIH and was the measure of one's success. In this sense, the situation was ideal, and at that time, unique. However, scientific leads were limited and the scientific community in general was extremely skeptical concerning a direct investment in therapeutic research. The fact that we were not limited by administrative overlay and fiscal restraints was good in one sense, but it meant that there were no excuses for failure.

We decided initially to focus on quantitative aspects of the natural history of acute leukemia in children, and particularly on the quantitative impact of therapy. While some information was available from earlier studies, much of it was qualitative. For example, bone marrow examination, which addresses the central parameter of leukemia, was infrequently performed and not generally used as a criterion for response.

Quantitative Clinical Trials

In collaboration with Dr. Holland, and subsequently on a multi-institutional basis (Acute Leukemia Group B), a series of quantitative studies, addressing a sequence of hypotheses, were conducted; they led to major progress in the treatment of acute lymphocytic leukemia (ALL). The first study largely addressed the prospective construction of a quantitative experimental design involving precise objective criteria for selection of patients, specification of treatment strategy and tactics, randomization, stratification, and criteria for quantification of response (1). Many of the criteria for clinical trials developed by Doll for non-neoplastic diseases were adapted to cancer by Zubrod (2) and applied for the first time to neoplasia. Prospective input in terms of the design came not only from investigators directly involved with the chemotherapy of acute leukemia but also, for example, from biostatisticians, basic scientists working with leukemia models, and pharmacologists. In essence, this study initiated in 1955 for patients with acute leukemia, in parallel with a comparable study initiated at the same time for solid tumors, served as a model for the subsequent construction of quantitative clinical trials in cancer, and it continues to do so (1,2).

---

[1] Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

[*] *Reprint requests to:* Emil Frei, III, MD, Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney St, Boston, MA 02115.

Complete Remission

The first study included 6-mercaptopurine (6-MP) and methotrexate (MTX). Complete remissions (CR), as rigorously defined to include a transition from pretreatment bone marrow packed with leukemia cells to marrow in which no leukemia cells could be detected, occurred in 20% of the patients. While a number of prognostic variables for survival, such as pretreatment wbc count, were beginning to become evident, by far the most powerful discriminant was the attainment of CR (1,3). For patients who did not achieve CR, the median survival was 2–3 months, which was comparable to that in the era prior to chemotherapy; for patients who achieved CR, the median survival was in the range of 10 months, and the improved survival could be attributed quantitatively to time spent in CR. This initial observation, subsequently confirmed not only for leukemia but for a variety of neoplastic diseases, indicated that the first goal towards a major impact on survival of patients with neoplastic disease was the attainment of CR.

Increasing the CR Rate: Combination Chemotherapy

In view of the first study, our next goal was to increase the initial CR rate. Experimental studies and preliminary clinical studies suggested that the most promising approach toward accomplishing this might be combination chemotherapy. Accordingly, our second quantitative study, initiated in 1958, involved randomization of patients with acute leukemia to three treatment arms: (*a*) MTX, (*b*) 6-MP, and (*c*) 6-MP plus MTX (4). Patients receiving a single drug initially were crossed over to the opposite drug upon relapse. Thus, one could quantitatively assess the CR rate and duration from the individual agents as compared to their combination. One could also assess the effect of prior exposure to one agent on the second, in a cross-over design, thus evaluating the relative merits of sequential versus concurrent combination chemotherapy. This study was based in part on the demonstration by Law (5) that antipurines plus MTX were moderately synergistic in L1210 mouse leukemia, and that 6-MP-resistant tumors were collaterally sensitive to MTX, presumably because such tumors cannot salvage purines. The experimental design was specifically constructed in a cross-over fashion to allow for determination of collateral sensitivity. It was found that the combination produced a CR rate that was somewhat superior to that of either agent alone (45%). Because both 6-MP and MTX produced dose-limiting myelosuppression, it was necessary to decrease the doses of each agent in the combination to 50%–60% of that employed when the agents were used alone. It was essential, therefore, to determine the dose effect; that is, how much of a sacrifice in effectiveness such a dose reduction would render.

Controlled studies of the dose effect were initiated in patients with Hodgkin's and non-Hodgkin's lymphoma for MTX and the alkylating agent thiotepa (6,8). In these studies, all variables except dose were controlled. Patients were randomized to (*a*) full (standard) dose or (*b*) one-half that dose. It was found, as expected, that there was a very substantial reduction in toxicity with lower doses. Very importantly, it was also found that with full dose a 50%–60% response rate was achieved, whereas with one-half the dose that effect was almost completely lost, the response rates being in the range of 10%. This steep dose-response curve relationship was consistent with experimental in vivo studies and has generally, though not always, been found in prospective dose-response studies in other tumors sensitive to chemotherapeutic agents (9). Thus, combination chemotherapy was much more likely to prove effective if doses of the individual agents could be maintained, a situation made possible where qualitatively different dose-limiting toxicity obtained for the individual agents. This was possible for prednisone and 6-MP which, when combined at full doses, produced a CR rate of 82% (6), and particularly for the combination of prednisone and vincristine (introduced into the clinic by Karon at NCI in 1962). The vincristine-prednisone combination was nonmyelosuppressive and, in both the initial study and the subsequent confirming studies, produced CR in 90% of children with ALL (10). These CR rates were substantially higher than those predicted, assuming independent drug action, indicating that at full doses true therapeutic synergism was achieved (6).

This sequence of studies led to an increase in the CR rate (from 20% to 90%) as a result of combination chemotherapy. Insights and scientific work leading to and deriving from these observations had a major and lasting effect on cancer chemotherapy. The importance of combination chemotherapy is evident from the pragmatic fact that essentially all highly effective and curative therapeutic regimens for human cancer involve combinations of two, and usually of three or four, agents. A major basic rationale for combination chemotherapy is tumor cell heterogeneity. This is evident from cytokinetic and cytogenetic studies, as well as from studies of antigen expression, hormone receptor expression, and especially the variability in response to chemotherapeutic agents on the part of tumor cells isolated from a given tumor. Such heterogeneity implies multiple targets and, therefore, multitargeted (combination) therapy. This heterogeneity is probably the primary basis for the selection of drug-resistant cell lines, and experimentally such resistance can be markedly reduced by the use of combination chemotherapy. Other rationales for combination chemotherapy include combined, sequential, or concurrent attack on biochemical pathways; the manipulation of drug transport; the use of metabolites to rescue normal, and hopefully not tumor, cells from antimetabolite effect; other approaches to metabolic modulation; and cytokinetic approaches involving cell synchrony and recruitment. The many and developing reasons for combination chemotherapy at basic and clinical levels have been reviewed (11-13).

Prolonging the Duration of CR

In spite of the achievement of a high CR rate, all patients eventually relapsed, usually within a matter of a few months, and the suggestion of clinical drug resistance became evident. The nature of relapse was studied. It usually involved the bone marrow and was often associated with symptomatology comparable to that which characterized the onset of the disease in the individual patient. Subsequently, with increasing perturbation of the disease by treatment, altered relapse patterns occurred (*see* below). Cytogenetic studies indicated that some 60% of patients with ALL had clonal disease initially, as evidenced by specific aneuploidy and markers; it disappeared during remission, and the same cytogenetic abnormality returned with relapse. Assuming that the cytogenetic abnormality marks the neoplastic cell, this confirmed the disappearance of neoplastic cells during CR and indicated that the pathogenesis of relapse was due to recurrent disease emerging from a microscopic tumor burden and not due to reinduced (new) disease. The generally exponential distribution of the relapse curve was also consistent with this conclusion. Therefore, a series of hypotheses concerning microscopic disease were developed, and clinical studies were conducted to define and control microscopic disease (14,15).

The first question was whether, once patients achieved a CR, an active agent continued during remission would in fact prolong the duration of remission. We found that 6-MP was markedly and significantly superior to a placebo in prolonging the duration of CR. Thus, it was active against microscopic disease. This study was the first quantitative "adjuvant" study, where CR is produced (either by chemotherapy or by surgical removal of the primary) and systemic treatment is employed to control microscopic disease (16).

Several quantitative studies conducted in patients in CR indicated that MTX was probably the best single agent. Experimental studies, particularly by Goldin et al (17) in transplanted mouse leukemia, indicated that for many agents (especially MTX), intermittent treatment was superior to continuous treatment. We were also influenced by studies in choriocarcinoma, where intermittent courses of MTX were curative (18). Accordingly, in the study involving remission induction with vincristine and prednisone, patients in CR were randomized to receive MTX twice weekly or to receive daily MTX, at doses which produced comparable toxicity. Intermittent MTX was significantly superior (median duration of CR, 9 months, versus 4 months for daily treatment) (10). The basis for the superiority of intermittent MTX was thought at the time to be cytokinetic, but more recent studies involving induction of resistance to MTX in mammalian cells suggest that resistance can be produced more readily by continuous, as compared to intermittent, exposure.

The dose effect of antitumor agents in lymphoma has already been underscored, and this was also found to be true in two comparative studies of combination chemotherapy in patients with ALL in remission (19,20).

Finally, as with CR induction, combination chemotherapy delivered during remission was found to be superior in prolonging the duration of remission. The combination of 6-MP given daily, with MTX given twice weekly, or once weekly, was found to substantially prolong the duration of systemic remission (21).

It was observed that the best agents for CR induction differed from the agents which were most effective for treatment during remission. Thus, vincristine plus prednisone was the optimal combination of two agents for CR induction, but when these agents were continued in remission they did not prolong the remissions (median, 2-3 months). In contrast, the combination of 6-MP and MTX was not particularly effective in inducing CR, but when employed during remission substantially prolonged the duration. Superiority of combination chemotherapy in prolonging the duration of remission was thought to relate, at least in part, to preventing the emergence of drug-resistant lines, as exemplified by the treatment of infectious diseases such as tuberculosis. A number of additional approaches, including rotational chemotherapy, intermittent "reinforcement" of antimetabolite chemotherapy by vincristine and prednisone, and the sequencing of multiple agents, were employed in an effort to prolong remissions. However, while these approaches significantly prolonged the duration of CR (control of microscopic disease), another obstacle to cure was emerging.

Meningeal Leukemia

Meningeal leukemia was rare prior to 1955, but as the proportion of patients entering CR increased substantially and the duration of systemic CR lengthened, the incidence of meningeal leukemia as the initial manifestation of relapse increased to the point where it occurred in 50%-60% of the patients. Meningeal leukemia consists of sheets of leukemic cells lining the meninges of the central nervous system, impeding egress of spinal fluid, and thus increasing intracranial pressure, with resultant headaches, changes in vital signs, and cranial nerve palsies. This occurs at a time when the patient is systemically in CR, as evidenced by normal bone marrow. It can be proven unequivocally by the demonstration of leukemia cells within spinal fluid obtained by lumbar puncture. Rall et al (22) demonstrated in pharmacologic studies that many of the antileukemic agents were largely excluded from the central nervous system, that is, did not pass the blood-brain barrier. MTX injected intrathecally had been demonstrated to be effective in decreasing pleocytosis and cerebrospinal fluid pressure and in improving symptomatology in such patients, but generally for a relatively brief period of time (23). Clinically evident meningeal leukemia was usually followed shortly by systemic relapse, suggesting that drug-resistant clones, selected in low drug concentrations in the central nervous system and capable of adapting to higher concentrations in the systemic circulation, were responsible for the sequence from meningeal to systemic relapse.

An elegant experimental model of meningeal leukemia in the mouse was developed and provided evidence supporting the following pathogenesis and approach to treatment. Early in the development of the leukemia process, before treatment is initiated, microscopic involvement of the central nervous system has occurred in most patients. With the application of systemic treatment, the systemic leukemia burden is controlled and the patient enters CR. However, the microscopic burden in the central nervous system is in a pharmacologic sanctuary and progresses to the point where eventually the clinical syndrome of meningeal leukemia emerges. This is consistent with the fact that the risk of developing meningeal leukemia increases linearly with time spent in CR (24).

The above suggested that therapeutic approaches conducted early in CR, designed to eradicate microscopic leukemic involvement of the central nervous system, would decrease the risk of development of overt meningeal leukemia. In a quantitative study, it was demonstrated that intrathecal MTX, given early in CR, significantly decreased the risk of meningeal leukemia (25). However, pharmacologic studies in primates demonstrated that the lumbar intrathecal administration of drugs, while providing good concentrations over the lower reaches of the central nervous system, failed in many instances to reach significant concentrations over the cerebral hemispheres (26). Accordingly, a series of elegant studies by Aur et al (27,28) and Pinkel (29) at St. Jude Hospital demonstrated that brain irradiation at relatively low doses, combined with lumbar intrathecal MTX, reduced the incidence of meningeal acute leukemia to $< 10\%$.

Curative Treatment of ALL

The sequence of studies described above, as well as cytokinetic modeling, suggested that the cure of ALL in children might be within reach, and prospective studies designed with curative intent were undertaken. The first of these was the VAMP program, introduced in 1961 (6,30). The four-drug combination involved vincristine and prednisone, given at full doses, with MTX (amethopterin) and 6-MP. Each course lasted 10 days, with a 2-week interruption; a total of five courses were given. Improved supportive care, in the form of platelet transfusions, antibiotics, and wbc transfusions, was employed. The VAMP program incorporated many of the aforementioned strategies including maintenance of the steep individual dose effect; change from the combinations in a sequence of two and two drugs to the concurrent use of four agents (intensive combined "up-front" therapy) in an attempt to maximize the rate of cytoreduction and to minimize the potential for the emergence of drug-resistant cells; and a sufficient interval between courses to allow for host recovery. Variations of this theme included the same agents given in a rotational fashion or for 12, rather than five, courses (BIKE and POMP programs) (31). Of the 64 patients treated with these programs, many had prolonged survival, and 10% were cured (6,30,31,57). The impact of these trials and the aforementioned strategic principles on cancer chemotherapy generally was major. However, the failure to include central nervous system prophylaxis (not critical for the treatment of Hodgkin's disease, for example, but essential for the treatment of ALL) limited the cure rate of the VAMP and related programs.

However, the optimal use of three principles led to cure rates of 40%: (*a*) CR induction with vincristine and prednisone; (*b*) central nervous system prophylaxis with intrathecal MTX and central nervous system irradiation; and (*c*) treatment during CR with intermittent MTX and daily 6-MP. This was demonstrated most convincingly initially by Pinkel et al at St. Jude Hospital, by Holland et al of the Cancer and Leukemia Group B (CALGB), and by Clarkson et al of Memorial Sloan-Kettering (29,32,33). While there have been a number of tactical variations and finally improvements on this theme, the essential strategy remains intact.

Experimental Cytokinetic Models: Application to ALL

In the early 1960s, Skipper and his associates (34) initiated a series of studies addressing the quantitative biology of leukemia in mice and its perturbation by chemotherapy. These studies have had a profound impact on our understanding of, and approaches to, therapeutic research in the clinic, initially with respect to ALL and subsequently with respect to all cancer. The first generalization, which they derived and demonstrated in a number of experimental in vivo studies, was the phenomenon of the first-order kinetic effect of chemotherapeutic agents. In summary, this states that for a given treatment it is the fractional reduction of tumor cells which is constant, not the absolute reduction. This constant fractional kill is independent of tumor burden (34).

Employing the Chalkley count technology and appropriate calculations, it was determined that patients with clinically overt ALL have a leukemia body burden of leukemia cells in the range of one trillion (10^{12}) (6). It is assumed that curative treatment requires the elimination by chemotherapy directly (presumably with some host help) of the entire leukemia burden. This is consistent with some of the transplanted mouse leukemias, where a single cell is capable of ascending to overt disease (35). CR induction therapy destroys an estimated kilogram of tumor and eliminates all clinical and laboratory evidence of leukemia. While this involves a $\geqslant$ 99% (2-log) reduction in the number of leukemia cells, it is only the beginning of successful treatment, considering the first-order kinetic concept. Thus, it takes just as much treatment to effect a 99% reduction from 10^{12} to 10^{10} cells (1 kg of tumor) as it does to effect a 99% reduction from, for example, 10^6 to 10^4 (< 1 mg of tumor). Hence, the achievement of a CR represents the initial step only, and continued treatment for a significant period of time after remission is essential for leukemia cell eradication. This model served as a frame of reference for treatment strategies and had major heuristic value. Its major assumption (leukemia cell homogeneity) was invalid, and the model has recently been modified to adjust particularly for cytokinetic heterogeneity and resistance mutation.

A major need in evaluating treatment is a method for determining the magnitude of cytoreduction during remission. For this, we turned to experimental models. Skipper had demonstrated, again for L1210 mouse leukemia, not only that time to death was a precise measure of the leukemia cell inoculum but also that time to death following treatment was a precise measure of the number of leukemia cells persisting at the end of treatment (6,34). This suggested that the duration of CR following cessation of therapy (duration of unmaintained remission) might correlate inversely with the number of leukemia cells persisting at the end of treatment and thus provide an estimate of the magnitude of leukemia cytoreduction. To apply this more quantitatively to the clinic, it was necessary to know the doubling time of leukemia cells after cessation of treatment. In a study of 17 patients through early and late bone marrow relapse following unmaintained remission, it was found that the median doubling time of leukemia cells in the marrow was 4 days (6). This was reasonably consistent with ^{3}H-thymidine studies of the kinetics of human ALL cells (58). Thus, in the hypothetic situation where the leukemia burden was reduced to one cell, the time to relapse was calculated as $1 \times 2^n = 1 \times 10^{12}$ (number of leukemia cells at relapse); $n = 41$ (number of doublings), 41×4 (doubling time in days) = 164 days to relapse from a single cell (6). The initial application of this program suggested exciting and consistent results. After CR induction by vincristine or prednisone (no maintenance treatment), relapse occurred in 40-50 days, suggesting a 2-4-log reduction in the leukemia burden. With the VAMP program the median time to relapse after cessation of treatment was 140 days, which was consistent with a 10-log reduction in leukemia burden, with three long-term survivors. However, subsequent studies involving longer durations of treatment (for example, the POMP program involving 12 months of four-drug treatment) produced an unmaintained remission lasting 220 days. This problem, which presumably related to heterogeneity, plus the emergence of meningeal leukemia, which when controlled was followed by a substantial increase in cure rate, diverted our attention from this approach. However, several years later when cure rates in the range of 50% were established, the general applicability of the model (appropriately modified) emerged.

While treatment prior to 1963 was often continued indefinitely in remission, it became apparent, particularly when higher cure rates were achieved, that this was neither practicable nor reasonable, considering the cytokinetics. Also, studies indicated that drug resistance was a ubiquitous problem and that it was unlikely, particularly with the antimetabolites, that drug-resistant clones would not emerge by 4 years of treatment, if not substantially before. As a result, in the curative regimens indicated above, treatment was discontinued at 2-3 years, and this was not associated with an increase in relapse rate (36). It was found that 10%-20% of patients in continuous CR, for whom treatment was stopped at 3 years, would subsequently relapse. Of the patients destined to relapse, 90% did so within 1 year, and most of these relapsed within the first 6 months. Indeed, the median time to relapse for relapsing patients in that setting was 5-6 months. Assuming that the patients who did not relapse had no leukemic cells and that the small proportion who did relapse had a marked reduction in the number of leukemia cells, the temporal relapse pattern in such patients is reasonably consistent with the cytokinetic model described above.

Lymphoma

The development of the treatment strategies for ALL have been applied with appropriate modification and success to a number of neoplastic diseases through the present time. The first, and most immediate, application was to Hodgkin's disease.

In a major, quantitative, comparative study initiated in 1960, it was observed that the available effective drugs (vinblastine and the alkylating agents) employed independently produced tumor regression in 50%-60% of patients, but CR was achieved only in approximately 10% (37). Moreover, in the absence of continuing treatment, such regressions were short, with median durations in the range of 2-4 months. The effect of dose was quantitatively studied in Hodgkin's disease, and found to be of major importance. The observations that MTX had activity in Hodgkin's disease comparable to that reported for the known active agents (7) and, in 1962 and early 1963, that vincristine not only was highly active for the treatment of Hodgkin's disease but also was nonmyelosuppressive set the stage for a combination-chemotherapy approach to patients with disseminated Hodgkin's disease (38). Instead of a series of studies involving two, and then more, agents, the principles established in ALL made it reasonable to employ all four known active agents. This was supported by the fact that two agents (vincristine and prednisone) were nonmyelosuppressive and could be employed at full doses, and the other two (MTX and cyclophosphamide [CY]) at only slightly compromised doses. Experimental and clinical evidence that intermittent treatment was superior to continuous treatment led to the employment of these four agents (MOMP) in intermittent courses of intensive treatment. The development of supportive care for myelosuppression (*see* below) allowed us to proceed with some security. Thus, the protocol for the MOMP program was written, and the first patient entered on protocol in 1963. It should be emphasized that the protocol was designed with curative intent. Thus, it was possible to cure patients with ALL with four agents having individual activity against ALL that was comparable to the activity of the four agents against Hodgkin's disease. Moreover, the tumor burden for disseminated Hodgkin's disease, while difficult to calculate, was estimated at a maximum of 10^{11} (1 log less than for ALL), primarily because diffuse marrow involvement rarely occurs in Hodgkin's disease. Moreover, it is quite probable that the proportion of Hodgkin's neoplastic cells within a given tumor represents a small fraction of the total number of cells. Finally, considering the cytokinetics, if each agent individually had the potential for a 2-3-log reduction, the four together at full or nearly full doses might be expected to render a Hodgkin's cell reduction that approximated the tumor burden.

While it took some 7 years to develop sufficient follow-up to demonstrate with reasonable security that some 40%-50% of patients were cured with this approach, it was clear from the beginning that a major advance had been achieved in the treatment of Hodgkin's disease. With single agents, CR occurred in 10%-20% of patients, tumor regression developed slowly, and remissions were of short duration. With MOMP, in contrast, tumor regression occurred with marked rapidity, such regressions were complete in 60%-70% of patients, and they were durable. In 1964, MTX was replaced by procarbazine because of developing evidence, largely in Europe, that procarbazine was the single most effective agent for the treatment of Hodgkin's disease (39-42).

These results have stood the test of time and have been extended in terms of demonstrations (*a*) that posttreatment staging was crucial to treatment strategy; (*b*) that pretreatment sites of bulk disease were high-risk sites of relapse; (*c*) that combination chemotherapy integrated with radiotherapy had important implications for intermediate stages of Hodgkin's disease and for bulk disease; (*d*) that the development of additional agents, and finally the ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) program, provided an alternative for treatment and the potential of alternating non-cross-resistant combinations; and (*e*) that the treatment strategy for Hodgkin's disease, appropriately modified, was curative for diffuse non-Hodgkin's lymphoma (43). These and other therapeutic perturbations had much to do in the mid-1960s with the need for more definitive and aggressive staging in terms of extent of disease, and particularly for more rational hematopathologic classification, both of Hodgkin's disease and non-Hodgkin's lymphoma, that emphasized classifications correlating with prognosis. Hematopathology training programs and repository (quality-control) committees were accordingly developed in the mid-1960s.

Acute and Chronic Myelogenous Leukemia

While many of the early studies of ALL were paralleled by comparable studies in acute myelogenous leukemia (AML), progress proceeded slowly because the individual agents were much less active in patients with AML. Both of the acute leukemias, particularly AML, provided a major need for, and stimulus to, blood product supportive care (*see* below). Chronic myelogenous leukemia (CML) provided a unique model, and with the discovery of the Ph^1 chromosome which marked the neoplastic cell, afforded an opportunity to study this disease therapeutically and in relation to its pathobiology. With single-agent therapy, such as busulfan, it was found that a substantial proportion of patients entered CR clinically and hematologically, but the Ph^1 chromosome persisted in essentially all scorable metaphases in the marrow. This suggested that such remissions, in contrast to those with acute leukemia, represented a shifting down of the cellularity and an increase in maturation of the marrow cells, but that neoplastic cells persisted (44,45). This was in keeping with the fact that such remissions, while benefiting patients symptomatically, did not prolong survival (in total contrast with acute leukemia). The observation that essentially 100% of scorable metaphases contained the Ph^1 chromosome in patients in CR, where the erythroid component constituted 60%-70% of the marrow, was the beginning of the developing weight of evidence that the Ph^1 chromosome marks not only the myeloid cell but also the erythroid, megakaryocytic, and, as subsequently discovered, lymphoid cell lines; thus, the neoplastic event presumably involves a pluripotential stem cell (46). This observation was consistent with the fact that, while remissions could be achieved in patients in blast crisis, the Ph^1 chromosome almost invariably persists, indicating that, in contrast to AML, normal marrow stem lines were suppressed or absent and incapable of reconstitution, at least by the time of blast crisis. These findings suggested that intensive treatment early in stable-phase CML might sufficiently suppress the neoplastic cells to allow for reconstitution by normal bone marrow stem cells. Such attempts have so far been rewarded with only limited success. Assuming that pluripotent stem cells and all of the marrow cells are neoplastic, the more logical therapeutic strategy would involve bone marrow transplantation, and this has been attended by increasing success in recent years.

Solid Tumors

Prior to 1965, with the exception of choriocarcinoma, progress in therapy for the more common epithelial solid tumors was limited, primarily because of lack of effective agents. However, quantitative clinical trials provided increased attention and improved care for these patients with metastatic disease, and much was learned concerning the natural history of various forms of metastatic cancer. The alkylating agents and antimetabolites (particularly CY, MTX, and 5-FU) and perhaps the vinca alkaloids had moderate activity in breast cancer and limited activity in other tumors. Because of this and because of the strategies developed for ALL, a combination of CY, MTX, 5-FU, and vincristine (CMFV) was developed in 1963 and proved to have substantial activity in patients with metastatic breast cancer. This led to the initial proposals that strategies for chemotherapy for patients with leukemia and lymphoma be applied to patients with solid tumor with minimal residual disease (adjuvant chemotherapy) (47). However, the use of CMFV in breast cancer was "lost to follow-up" in 1965, and the study was published in retrospect in 1972 (48). Combinations involving CMF for breast cancer were independently rediscovered in the late 1960s.

Supportive Care

Both acute leukemia and its treatment produce myelosuppression as the major cause of morbidity and mortality. It was apparent that supportive care, in terms of the prevention and treatment of thrombocytopenic hemorrhage and granulocytopenic infection, was essential and should make possible a more effective treatment for these and other tumors. In a retrospective analysis of patients with thrombocytopenic hemorrhage receiving platelet transfusions, it was found that such transfusions were often effective, generally to the degree to which they increased the platelet count in the recipient. A randomized comparative study of thrombocytopenic hemorrhage demonstrated conclusively in a small number of patients that platelet transfusions were highly effective in controlling such hemorrhage (49). While the induction of allogeneic immunization and thus loss of effectiveness of platelet transfusions were found to occur in a matter of a few weeks in patients with aplastic anemia, the prediction that this would happen in acute leukemia was not validated, presumably because of the immunosuppression intrinsic to the disease and its treatment. Quantitative studies indicated that if platelet transfusions were used to maintain the platelet count at $> 20,000/\text{mm}^3$ during remission induction, hemorrhage was infrequent (50). Hemorrhage as a major cause of morbidity and mortality in patients during remission induction of acute leukemia was reduced from 60% to $< 20\%$ as a result of platelet transfusions (51). Importantly, such support allowed for more aggressive and therefore more effective chemotherapy, particularly for AML. With the control of hemorrhage, infections, which were always an important problem, became the overwhelmingly major problem with respect to morbidity and mortality during remission induction in acute leukemia. These were predominantly bacterial infections and could be quantitatively related to the magnitude and duration of granulocytopenia. Antibiotics had an increasingly major role in the control of such infections. For example, in the late 1950s, a marked increase in morbidity and mortality from *Staphylococcus aureus* occurred as a result of drug resistance. This problem was almost eliminated with the advent of the penicillinase-resistant semisynthetic penicillins. The development of carbenicillin, the aminoglycosides, amphotericin, and more recently the cephalosporins and the antiviral agents has had a marked impact on our ability to control and prevent the changing spectrum of infections.

In a pilot study, the buffy coat from a patient with stable-phase CML with a wbc count of $260,000/\text{mm}^3$ was transfused (8×10^{10} cells) into a child with ALL who had *Pseudomonas* sepsis due to marked granulocytopenia. One hour after transfusion, the granulocytes in the recipient had increased to $2000/\text{mm}^3$, with a half-time of approximately 6 hours. Importantly, there was prompt defervescence, blood cultures became negative, and the child survived an infection which had a $> 80\%$ chance of being fatal. The effectiveness of granulocyte transfusions so delivered was unequivocally demonstrated in the early 1960s. The dose of donor granulocytes was crucial. The range delivered was between 10^{10} and 10^{11}. For patients receiving 10^{11} granulocytes, an increment in the recipient of 1000–3000 cells occurred regularly, whereas for patients receiving 10^{10} donor granulocytes, the increment was marginal and not reproducible. This increment was measured at 1 hour, and the half-time was such that by 24 hours the granulocyte count had returned substantially to pretransfusion levels. Similarly, control of fever and/or infection was dose-related: $> 80\%$ of episodes treated with $\geqslant 8 \times 10^{10}$ granulocytes responded, whereas only 10%–20% of patients receiving $1\text{–}3 \times 10^{10}$ donor granulocytes responded (52,53).

An unanticipated observation was made. Several of the recipients had the expected 1-hour increase in circulating granulocytes, which, instead of abating, increased over a period of time, exceeding many weeks in several patients. Moreover, bone marrow examination at this time revealed hyperplasia, largely myeloid in constitution, instead of the anticipated hypoplasia. When cytogenetics were applied to such marrows, the Ph^1 chromosome was observed, indicating cytogenetically as well as hematologically and clinically that functioning bone marrow grafts had been accomplished. All of the recipients had advanced refractory disease, so that more definitive studies of the grafts and possible graft-versus-host disease were difficult to interpret (54).

The effectiveness of CML-donated granulocyte transfusions led to a major program designed to acquire granulocytes from normal donors. In collaboration with engineers at IBM, a continuous-flow centrifuge with a separate continuous collection for the buffy coat was developed (55). This equipment is now a part of most major blood banks, where it plays a major role in the acquisition of plasma and blood products. Unfortunately, the maximum number of granulocytes collected by this technique was in the range of 1×10^{10}, and even by increasing rouleaux formation with hydroxyethyl starch or by producing neutrophilia in the donor by prednisone or etiocholanolone, donor granulocyte numbers exceeding $2–3 \times 10^{10}$ were difficult to achieve. Considering the quantitative studies with respect to CML donation, it is not surprising that granulocyte transfusions in this range might be of limited effectiveness. A number of comparative studies were subsequently conducted, most of which demonstrated that granulocyte transfusions have a limited but statistically significant advantage over controls. Some investigators feel that this limited advantage is offset by the expense and potential toxicity of allogeneic granulocytes. Further research is directed towards increasing the granulocyte yield from the donor through bioengineering, increasing the effectiveness of individual granulocytes by the concurrent administration of opsonins (eg, for *Escherichia coli*), long-term marrow culture with the production of granulocytes, and possibly even returning to CML donors.

Protected Environment: Prophylactic Antibiotics

The risk of bacterial infections relates to the degree of myelosuppression and immunosuppression and also to the presence and particularly the magnitude of colonization (which occurs in inpatients receiving antibiotics, with pathology at potential entry sites). The tolerated dose of myelosuppressive chemotherapeutic agents was demonstrated to be substantially increased in gnotobiotic rodents, and dogs given oral prophylactic antibiotics have a substantial protection of their gastrointestinal tract from MTX toxicity. The immediate occasion for the initiation of the program was the acquisition by NIH of several "life islands," ie, beds with plastic bubbles with a double-door ultraviolet light passed through. An absolute (HEPA) filter system plus sterilization of diet and all other materials passed into the bubble precluded exogenous contamination. Endogenous decontamination was accomplished by oral, nonabsorbable antibiotics (an aminoglycoside, vancomycin, and nystatin), and by external aseptic techniques, including topical antibiotics, with emphasis on the oral cavity and intertrigenous areas. Quantitative cultures in the pilot patient indicated a marked reduction in bacterial contamination of these sites, including the gastrointestinal tract, and there was suggestive evidence in the initial studies for a decreased incidence of local and systemic infections (56). This study was initiated at NCI in 1964, and the more definitive studies, including the evolution towards laminar air-flow rooms, were continued after 1965 at NCI, the M. D. Anderson Hospital and Tumor Institute, and subsequently elsewhere.

Drug Development, Pharmacology, and New Agents

Drug development and pharmacology were increasingly emphasized in the intramural and extramural NCI and independent programs. The Medicine Branch collaborated in a number of studies involving important new agents, such as vincristine, vinblastine, mitoguazone, CY, 5-FU, dichloromethotrexate, and the nitrosoureas. These included not only clinical trials, but also pharmacologic studies, especially those of methotrexate with relation to the blood-brain barrier.

Final Remarks

A number of individuals made major contributions to the decade of progress for the Medicine Branch from 1955 to 1965. Dr. Zubrod's role has already been emphasized. Dr. Freireich was a creative participant in the acute leukemia studies and was the prime mover for the supportive care studies. Dr. Karon, the first pediatrician in the Medicine Branch, addressed the patient-family situation and was the first to emphasize the need for straightforward communication with parents and patients. He was also heavily involved in the acute leukemia studies, particularly with vincristine. Dr. Carbone contributed to the solid tumor and lymphoma studies, most particularly those with combination chemotherapy, and was primarily responsible for the CML programs. Dr. Brindley carried major clinical responsibilities. Dr. Perry played a significant role in the hematologic studies, particularly those relating to supportive care. One of the major assets of the Clinical Center was the annual acquisition of highly qualified clinical associates (fellows) with interest in research. Many of these associates acquired a permanent interest in, and commitment to, cancer research and today hold major leadership positions in cancer research around the country. Three of these people should be cited for highly important contributions during their tenure as clinical associates: Dr. Levin, who had an important role in supportive care, particularly granulocyte transfusion research; and Dr. DeVita, who, with his fellow clinical associate Dr. Moxley, shared responsibility with the senior staff for the conceptualization, implementation, and operation of the combination-chemotherapy program for Hodgkin's disease.

The Medicine Branch was an integral part of NCI and NIH and benefited enormously from interactions with the many clinical and basic scientists who made up that stimulating environment. Emphasis should be given to our interaction with the Pharmacology Branch, particularly Drs. Rall and Loo; and with the Metabolism Section under Dr. Berlin, Surgery under Dr. Ketchum, and Pathology under Dr. Thomas. Biostatisticians, including Drs. Gehan, Schneiderman, and Zelen, played a major role in the experimental designs, data management, and analysis. Many of the studies were made possible by the highly sophisticated cytogenetic studies of Dr. Whang. Finally, as already indicated, some of the studies were conducted as a part of the CALGB, under Dr. Holland, and the Eastern Cooperative Oncology Group, under Dr. Zubrod.

REFERENCES

1. FREI E, III, HOLLAND JF, SCHNEIDERMAN MA, ET AL. A comparative study of two regimens of combination chemotherapy in acute leukemia. Blood 13:1126-1148, 1958.
2. ZUBROD CG, SCHNEIDERMAN M, FREI E, III, ET AL. Appraisal of methods for the study of chemotherapy of cancer in man: comparative therapeutic trial of nitrogen mustard and triethylene thiophosphoramide. J Chronic Dis 11:7-33, 1960.
3. FREIREICH EJ, GEHAN E, SHULMAN D, ET AL. The effect of chemotherapy on acute leukemia in the human. J Chronic Dis 14:593-608, 1961.
4. FREI E, III, FREIREICH EJ, GEHAN E, ET AL. Studies of sequential and combination antimetabolite therapy in acute leukemia: 6-mercaptopurine and methotrexate. Blood 18:431-454, 1961.
5. LAW LW. Effects of combinations of antileukemic agents on an acute lymphocytic leukemia in mice. Cancer Res 12:871, 1952.
6. FREI E, III, and FREIREICH EJ. Progress and perspectives in the chemotherapy of acute leukemia. Adv Chemother 2:269-289, 1965.
7. FREI E, III, SPURR CL, BRINDLEY CO, ET AL. Clinical studies of dichloromethotrexate (NSC 29630). Clin Pharmacol Ther 6:160-171, 1965.
8. BRINDLEY CO, ET AL. Further comparative trial of thio-TEPA and mechlorethamine in Hodgkin's disease and melanoma. J Chronic Dis 17:19, 1964.
9. FREI E, III, and CANELLOS GP. Dose: a critical factor in cancer chemotherapy. Am J Med 69:585-594, 1980.
10. SELAWRY OS, HANANIAN J, WOLMAN IJ, ET AL. New treatment schedule with improved survival in childhood leukemia. JAMA 194:75-81, 1965.
11. HEPPNER A, and CALABRESI P. Heterogeneity of human tumors. Cancer Res 38:3758-3763, 1979.
12. FREI E, III. Combination cancer therapy: Presidential address. Cancer Res 32:2593-2607, 1972.
13. BLUM R, and FREI E, III. Combination chemotherapy. Methods Cancer Res 17:215, 1979.
14. ZUELZER WW, INOUE S, and THOMPSON RI. Long-term cytogenic studies in acute leukemia of children. The nature of relapse. Am J Hematol 1:143, 1976.
15. WHANG-PENG J, FREIREICH EJ, OPPENHEIM JJ, ET AL. Cytogenetic studies in 45 patients with acute lymphocytic leukemia. JNCI 42:881-897, 1969.
16. FREIREICH EJ, GEHAN E, FREI E, III, ET AL. The effect of 6-mercaptopurine on the duration of steroid-induced remissions in acute leukemia: a model for evaluation of other potentially useful therapy. Blood 21:699-716, 1963.
17. GOLDIN A, VENDITTI JM, HUMPHREYS SB, ET AL. Modification of treatment schedules in the management of advanced mouse leukemia with amethopterin. JNCI 17:293, 1956.
18. LEE MD, HERTZ R, and SPENCER DB. Effect of MTX therapy upon choriocarcinoma and chorioadenoma. Proc Soc Exp Biol Med 93:361, 1956.
19. PINKEL D, HERNANDEZ K, and BORELLA L. Drug dosage and remission duration in childhood lymphocytic leukemia. Cancer 27:247, 1971.
20. HOLLAND JF. Unpublished observation.
21. Acute Lymphocytic Leukemia. *In* Leukemia (Gunz FW, and Henderson ES, eds). New York, Grune and Stratton 1983, 4th ed, p 575.
22. RALL DP, STABENAU JP, and ZUBROD CG. Distribution of drugs between blood and cerebrospinal fluid: general methodology and effect of pH gradients. J Pharm Exp Ther 125:185-193, 1959.
23. WHITESIDE JA, PHILLIPS FS, STURGEON HW, ET AL. Intrathecal aminopterin in the neurological manifestations of leukemia. Arch Intern Med 101:280, 1958.
24. BLEYER WA. Central nervous system leukemia. *In* Leukemia (Gunz FW, and Henderson ES, eds). New York, Grune & Stratton, 1982, 4th ed, p 865.
25. FREI E, III, KARON M, LEVIN RH, ET AL. The effectiveness of combinations of antileukemic agents in inducing and maintaining remission in children with acute leukemia. Blood 26:642-656, 1965.
26. RIESELBACH RE, DICHIRO G, FREIREICH EJ, ET AL. Subarachnoid distribution of drugs after lumbar puncture. N Engl J Med 267:1273, 1962.
27. AUR RJA, SIMONE JV, HUSTU HO, ET AL. A comparative study of central nervous system irradiation and intensive chemotherapy early in remission in childhood acute lymphocytic leukemia. Cancer 29:381-391, 1972.
28. AUR RJA, SIMONE JV, HUSTO HO, ET AL. Central nervous system therapy and combination chemotherapy of childhood lymphocytic leukemia. Blood 37:272-281, 1971.
29. PINKEL D. The ninth annual David Karnofsky lecture: treatment of acute lymphocytic leukemia. Cancer 48:1128, 1979.
30. FREIREICH EJ, KARON M, and FREI E, III. Combination chemotherapy of childhood leukemia with vincristine, amethopterin, mercaptopurine, and prednisone (VAMP). Proc Am Assoc Cancer Res and ASCO 5:50, 1964.
31. FREIREICH EJ, HENDERSON ES, KARON MR, ET AL. The treatment of acute leukemia with respect to cell population kinetics. *In* The Proliferation and Spread of Neoplastic Cells, Baltimore, Williams and Wilkins, 1968, pp 441-452.
32. HOLLAND JF. Breaking the cure barrier. Karnofsky Memorial lecture. J Clin Oncol 1:75, 1983.
33. ______. *E. pluribus unum:* Presidential Address. Cancer Res 31:1319-1329, 1971.
34. SKIPPER HE, SCHABEL FM, and WILCOX WS. Experimental Evaluation of Potential Anticancer Agents. XIII. On the Criteria and Kinetics Associated with "Curability" of Experimental Leukemia. Cancer Chemother Rep 35:1, 1964.
35. FURTH J, and KAHN MC. The transmission of leukemia of mice with a single cell. Am J Cancer 31:276, 1938.
36. AUR RJA, SIMONE JV, HUSTU HO, ET AL. Cessation of therapy during complete remission of childhood acute leukemia. N Engl J Med 291:1230, 1974.
37. CARBONE PP, ET AL. Management of patients with malignant lymphoma: a comparative study with cyclophosphamide and vinca alkaloids. Cancer Res 28:811-822, 1968.
38. CARBONE PP, BONO V, FREI E, III, ET AL. Clinical studies with vincristine. Blood 21:640-647, 1963.
39. FREI E, III, DEVITA VT, MOXLEY JH, III, ET AL. Approaches to improving the chemotherapy of Hodgkin's disease. Cancer Res 26:1284-1989, 1966.

40. DEVITA VT, SERPIK A, and CARBONE PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 73:881, 1970.
41. FREI E, III, LUCE JK, GAMBLE JF, ET AL. Combination chemotherapy in advanced Hodgkin's disease—induction and maintenance of remission. Ann Intern Med 79:376-382, 1973.
42. DEVITA VT, JR, SIMON RM, HUBBARD SM, ET AL. Curability of advanced Hodgkin's disease with chemotherapy. Ann Intern Med 92:327-342, 1980.
43. DEVITA VT, CANELLOS GP, CHABNER BA, ET AL. Advanced diffuse histiocytic lymphoma, a potentially curable disease. Lancet 1:248, 1975.
44. CARBONE PP, TJIO JH, WHANG J, ET AL. The effect of treatment in patients with chronic myelogenous leukemia. Hematologic and cytogenetic studies. Ann Intern Med 59:622-628, 1963.
45. WHANG J, FREI E, III, TJIO JH, ET AL. The distribution of the Philadelphia chromosome in patients with chronic myelogenous leukemia. Blood 22:664-673.
46. FREI E, III, TJIO JH, WHANG J, ET AL. Studies of the Philadelphia chromosome in patients with chronic myelogenous leukemia. Ann NY Acad Sci 113:1073-1080, 1964.
47. FREI E, III. Selected considerations regarding chemotherapy as adjuvant in cancer treatment. Cancer Chemother Rep 50:1-8, 1966.
48. SHNIDER BI, BAIG M, SERPICK A, ET AL. J Clin Pharm 15:69-73, 1975.
49. FREIREICH EJ, SCHMIDT PJ, SCHNEIDERMAN MA, ET AL. A comparative study of the effect of transfusion of fresh and preserved whole blood on bleeding in patients with acute leukemia. N Engl J Med 260:6-11, 1959.
50. GAYDOS LA, FREIREICH EJ, and MANTEL N. The quantitative relation between platelet count and hemorrhage in patients with acute leukemia. N Engl J Med 266:905-909, 1962.
51. HERSH EM, BODEY GP, NIES BA, ET AL. Cause of death in acute leukemia: a 10-year study of 414 patients. JAMA 193:105-109, 1965.
52. FREIREICH EJ, LEVIN RH, WANG J, ET AL. The function and fate of transfused leukocytes from donors with CML in leukopenic recipients. Ann NY Acad Sci 113:1081-1089, 1964.
53. FREIREICH EJ, MORSE EE, BRONSON W, ET AL. Transfusion of granulocytes from donors with CML to leukopenic recipients. *In* Proceedings of the 19th Congress of the International Society of Hematology 1:549-557, 1962.
54. LEVIN RH, WANG J, CARBONE PP, ET AL. Erythroid homografts following leukocyte transfusion in a patient with acute leukemia. Blood 26:587-609, 1965.
55. FREIREICH EJ. Continuous flow in vivo blood cell separator. Lab Management 6:20-23;43-48, 1968.
56. SCHWARTZ A, COLVIN OM, HIMMELSBACH CK, ET AL. Mechanical isolation and prophylactic antibiotics for cancer chemotherapy. Clin Res 13:48, 1965.
57. LEVENTHAL BG, LEVINE AS, GRAW RG, JR, ET AL. Long-term second remissions in acute lymphocytic leukemia. Cancer 35:1136-1140, 1975.
58. MAUER AM, EVERT CF, JR, and LAMPKIN BC. Cell kinetics in human acute lymphoblastic leukemia: computer simulation with discreet modelling techniques. Blood 41:141, 1973.

Cancer Surgery: A Commentary

Bernard Fisher[1,*]

When consideration is given to progress made during the past decade or so relative to the treatment of primary cancer (solid tumors), attention is usually focused upon the putative contributions of medical and radiation oncology. Chemotherapeutic, endocrinologic, and immunologic manipulations attract positive interest and are deemed promising for future patient benefit. By contrast, the surgical approach to cancer treatment is considered by many, particularly those in other disciplines, to be anachronistic and is often the object of derision. The impression is that there are no new frontiers in surgery which, by their exploration, can impact upon the treatment of cancer. At present, uncertainty exists regarding the appropriate use of this intervention modality, and while such uncertainty is currently most apparent in the treatment of breast cancer, it relates to the surgical management of other solid tumors as well. It is vexing that there is so little awareness by physicians, patients, and the public as to why this change in attitude toward surgery has taken place; reaction to the change is often distressingly naive. For many people, it is difficult to accept that a discipline which not so long ago afforded standard treatment in a structured and methodic fashion should now be reduced to justifying its position in the family of modalities available for the therapy of cancer. The diversity of opinion regarding the primary treatment of breast cancer, for example, is too often looked upon by patients, consumer groups, public officials, and physicians with strong biases as an expression of medical incompetence which requires correction by legislation, populist action, and education. For surgeons faced with a need to make immediate decisions regarding the care of individual patients, the nonuniformity provides an imprimatur for improvisation or for acquiescing to consumer demands, ie, being dictated to by the "marketplace." Among many surgeons in positions of influence, the changing role of surgery in oncology is interpreted as being related to the fact that while medical oncology and radiation oncology have prospered because of strong leadership and support by the National Cancer Institute, surgical oncology has languished due to neglect; they believe that to regain its eminence an "affirmative action program," which provides funds and better recognition of surgical oncology as a defined discipline, is required.

Over the years, I have repeatedly presented my own perception of the reasons for and the significance of the changing role of surgery in the treatment of cancer (1-5). I have provided evidence to indicate that there are indeed scientific reasons for the change and that it has been entirely fortuitous that "nonscience" (anecdotalism) has made a contribution. It is my view that an altered comprehension of the biology of cancer acquired over the past two decades from the results of laboratory and clinical investigations which embodied the application of the scientific method is the factor which is primarily responsible for the formulation of a new basis for cancer surgery. As frequently occurs during a period when there is a transition of concept, uncertainty prevails and will remain until a new rationale for the employment of cancer surgery is firmly entrenched.

I have indicated that a proper portrayal of the developmental pathways of a physical science describes the transition from paradigm to paradigm occurring as the result of revolutionary scientific accomplishments and that a similar approach may be useful to depict the development of various components of the biologic sciences as well (4). Since the discipline of surgery consists of more than a mastery of technical skills and ought no longer be defined as the "art, practice or work of treating diseases, injuries or deformities by normal operation or instrumental appliances" but rather should be considered a branch of knowledge governed by facts or principles gained by systematic study, I have attempted to discern whether the developmental pathway of the surgical therapy of cancer has evolved and continues to progress as the result of paradigmatic changes—revolutions—which are products of scientific investigation, or whether it is a consequence of congeries of observations made in random or near-random fashion.

This brief commentary provides an opportunity to recount a few of my thoughts about cancer surgery which have evolved over more than two decades as a result of involvement with the discipline at both the laboratory and the clinical level. It considers the basis for cancer sur-

---

[1] Department of Surgery, University of Pittsburgh, PA.

[*] *Reprint requests to:* Bernard Fisher, MD, University of Pittsburgh, 3550 Terrace St, 914 Scaife Hall, Pittsburgh, PA 15261.

gery in the past, factors which have modified and are modifying those principles, contributions made by surgery to a better understanding of cancer, the likely role of operation for the management of cancer in the future, and my perception of what constitutes a surgical oncologist. Because breast cancer has so often served as the study model, reference will frequently be made to that tumor. It is emphasized, however, that within the context of this discussion, findings obtained with that model are very likely to have relevance to all solid tumors.

Evolution of the Operative Treatment of Cancer

Origins of the Halstedian Hypothesis

All operations require a well-founded reason for their use. Just as repair of a hernia is based on anatomic considerations and operations for duodenal ulcer on physiologic principles, so must there be a firm rationale for the surgical management of cancer. In tracing the evolution of cancer surgery through the ages, it is of importance to know how those people who formulated and influenced treatment at a particular time in history conceived of the disease, for it was (and still is) their conceptions that provided the reasons for the therapy employed.

Hippocrates, 2400 years ago, described many forms of cancer and originated the term "carcinoma." Unfortunately, there is little or no information to indicate his perception of the disease. Galen, in the second century AD, depicted cancer as a systemic disease related to an excess of black bile and, therefore, beyond cure by operation. His teachings held sway until the 18th century and, consequently, until that time there existed no firm justification for the use of operation in the treatment of cancer. The first surgeon to consider cancer a local and curable disease is not known; several in the 18th century made an effort to develop a rationale for cancer surgery. Valsalva (1704) discarded Galenic teaching and espoused the belief that cancer spread by lymphatics to regional nodes and that it tended to recur. Le Dran (1757) and Morgagni (1769) assumed that cancer was a local disease that was curable if found sufficiently early.

The first true paradigm for cancer management was formulated almost 100 years ago. No one was more influential in that synthesis than the American surgeon William S. Halsted. While his name is most closely associated with the operation for breast cancer, ie, radical mastectomy, the same precepts promulgated by him as justification for carrying out that procedure have served as an enduring basis for all cancer surgery and for the use of postoperative radiation as well. To understand Halsted's rationale for the type of surgery he advocated, it is important to appreciate his concept of the biology of cancer, particularly how he thought tumors disseminated. Information from several diverse investigations conducted around 1880 led to the definition of the anatomic and mechanistic principles of cancer surgery which became recognized as "halstedian." Goldman and Schmidt demonstrated that cancer cells in blood excited thrombosis. They concluded that the thrombi destroyed or rendered such cells harmless. That finding, as interpreted by Halsted, minimized the importance of the blood vascular system as a mechanism for the development of metastases. The report of Handley that tumor cells spread along lymphatic pathways by direct extension rather than by embolization also played a seminal role in the formulation of the halstedian hypothesis of cancer management. Halsted was convinced that there was contiguity between a primary tumor and its metastasis, even when one occurred remote from the other, eg, a breast cancer and a metastatic lesion in the femur. Studies by Virchow indicating that regional lymph nodes were effective filters which could serve as a barrier to tumor cell spread further influenced the evolution of the halstedian hypothesis. Another precept that was in harmony with the hypothesis and gave rise to "modern" cancer surgery was the concept that a growing tumor remains localized at its site of origin for a period of time, but at some instant during its growth, tumor-cell invasion of lymphatics and dissemination to regional nodes take place. After a further interval, during which the tumor is local-regional only and increases in size, systemic dissemination occurs.

As a consequence of the above considerations and in keeping with the understanding of the mechanism of cancer metastases at the time, there arose an "anatomic" basis for cancer surgery. The "proper" cancer operation consisted of removal of the primary tumor together with regional lymphatics and lymph nodes by en bloc dissection. Since it was deemed that there was a certain "orderliness" about tumor spread and that clinically recognizable cancer was in many instances a local-regional disease, it was considered that tumors would be more curable if surgeons would only be more expansive in their interpretation of what constituted the "region" and if, above all, they utilized better technic so that they could eradicate every cancer cell. Local-regional recurrences were more often than not considered to be the result of inadequate application of surgical skill rather than a manifestation of systemic disease. There was hope that "one more lymph node dissection would cure more cancers." Radical cancer surgery based on those anatomic considerations persisted for 75 years and still endures to varying degrees in the minds of many people.

With the advent of better supportive measures to improve patient survival, ie, blood transfusions, anesthesia, correction of physiologic deficits resulting from operation, and development of technical skills, super-radical cancer surgery came into being. Not too long ago (and still occasionally) such procedures as extended radical mastectomy, hemipelvectomy, forequarter amputation, partial evisceration, and even hemicorpectomy were carried out and written about.

Other nuances of surgical technic, for the most part mechanical and simplistic in concept, have been introduced from time to time by surgeons in an attempt to "trap" and eliminate the "stray" tumor cell. A prime impetus to such procedures was the demonstration of tumor cells in the blood of patients undergoing operation. To meet the challenge of those findings, there followed the "no-touch technic," the employment of "preliminary venous ligation," the use of "taping" of segments of intestine above and below tumors, the washing of operative wounds, and/or the intraluminal instillation with a variety of cytocidal agents. The rationales for the use of regional perfusion of anticancer agents, the use of regional radiation therapy, and the most recent use of regional hyperthermia embody halstedian principles.

Several comments regarding the halstedian hypothesis are appropriate. First, it is important to stress that it was synthesized from principles which were considered at the time to be "scientific." Theories which have been replaced are not unscientific. If the term "nonscientific" or "error" is assigned to outdated concepts, then scientific knowledge of today is apt to be judged in the future as nonscience and error as well. Second, probably because it was not replacing an already entrenched paradigm and because for many years there were few challenges to it arising from the results of laboratory and clinical studies, the hypothesis was accepted without test of its validity.

That the halstedian hypothesis should have resulted in a paradigm for cancer management which endured for nearly three-quarters of a century seems remarkable. Careful consideration indicates that there was indeed no reason for anything but a prolonged tenure. The study of paradigms is what prepares a student for membership in the particular professional community in which he will practice. The halstedian paradigm became rapidly and universally accepted because of lack of competition. Indoctrination of others was carried out by ardent and dedicated proponents. The subsequent practice of the novice was among other practitioners who shared the same rules and standards—the same paradigm—for practice. With such a consensus, disagreement over fundamentals would have been unlikely, and the theory thus remained essentially unchallenged. Moreover, the paradigm remained intact because there were few substantive challenges to it arising from the results of laboratory and clinical investigation. Little had changed in the perception of the process of metastases, that aspect of tumor biology which supplies much of the rationale for cancer surgery. While by then it was accepted that lymphatic spread occurred by embolization instead of by direct extension, it was agreed that the bloodstream was an important pathway of tumor dissemination, and certain aspects of the process had been clarified, the overall concept remained unaltered. The idea prevailed that lymph-borne tumor cells had one destination, lymph nodes, that tumor cells in blood lodged in the first capillary bed they encountered, and that there was an orderly pattern of dissemination based upon temporal and mechanical considerations. The only debate related to whether patterns of distant metastases were due to specific properties of various tissues, ie, the "seed-soil" hypothesis first promulgated by Paget in 1889, or to anatomic and mechanical factors. Findings from studies could not be unified to produce a competing hypothesis.

Clinical Challenges of the Halstedian Hypothesis

Beginning in the 1960s, challenges to the halstedian paradigm for cancer surgery began to emerge. They arose not only from the clinic (4) but also from the laboratory (3). In the clinic, breast cancer served almost entirely as the test model, probably because of its frequent occurrence, accessibility for evaluation, and emotional impact. Thus, the clinical challenges of the halstedian hypothesis were mainly directed toward the radical mastectomy, which expresses its principles.

The clinical challenges should be put into proper perspective. Those challenges were not directed toward the principles upon which the hypothesis was based but rather were more concerned with whether or not the radical operations employed were adequately embodying those principles. If removal of the entire lymphatic drainage area was of paramount importance, then the radical operation for breast cancer, for example, was inadequate, since lymph nodes other than those in the axilla were frequently involved with tumor. A flurry of anecdotal reports appeared which narrated personal experiences with *extended* radical mastectomy. In 1970, after an extensive evaluation of the reports, I concluded that the material presented had not demonstrated that the extended radical procedure was or was not more efficacious than the conventional radical mastectomy. Despite large numbers of patients in many of the studies, the evaluations were inadequate to test the worth of the super-radical operation. Nothing came from those reports which could provide a basis for challenging halstedian principles. Nothing arose from them which added to comprehension of the disease. There was no research component in those efforts. Unfortunately, the same may be said relative to the accomplishments and contributions made by other operations of the same genre directed toward the treatment of other tumor types. Considerable physical and mental trauma was inflicted without such dividends.

At the same time that more encompassing operations were being carried out, there were reports of experiences with less extensive operations. The deviation from radical mastectomy toward lesser operations was not because the halstedian hypothesis was being displaced by new facts or concepts. The change was primarily due to dissatisfaction with the results of the operation. The considerations of McWhirter, who in 1948 reported his results with simple mastectomy combined with postoperative radiotherapy, best exemplify that attitude. He adopted his "new" therapeutic regimen because of concern that radical operations were not radical enough. They failed to get rid of all tumor tissue in the operative area, and "at the time of operation tissues actually invaded by tumor must often be divided." It was his opinion that, as a result of this trauma, malignant cells would have an increased tendency to disseminate to other sites. Although he appreciated the possibility that such cells could still be liberated from the area of operation when a simple mastectomy was performed, he was of the opinion that they could be trapped by the intact barrier of the axilla. Moreover, since wound healing was likely to take place more rapidly after simple than after radical mastectomy, radiotherapy could be applied with less delay, thus reducing the interval during which cells could be disseminated to distant sites. Those views were in keeping with the mechanistic approach toward both tumor dissemination and eradication. They were not challenging the prevailing paradigm.

Again, as with super-radical mastectomy, aside from a demonstration of personal conviction, the plethora of anecdotal data was nondefinitive. It failed to demonstrate unequivocally whether simple mastectomy was a procedure that should or should not supplant the radical operation. The clinical tests were, almost without exception, "no contests." Only Crile (6) proposed a new concept which was based on biologic considerations. Influenced by the findings of others which indicated the importance of regional lymph nodes in the immunologic response of a host to a transplant, he postulated that there might be value in not removing regional lymph nodes.

While most clinicians were debating the relative clinical merits of radical, super-radical, and simple mastectomy, a few were reporting their experiences with modified radical mastectomy or local excision and radiation. The rationale for that operation was based on the consideration that since the radical mastectomy left behind internal mammary nodes it was not adequate. The use of irradiation following modified radical mastectomy would be more rational, since the internal mammary nodes would thus be treated.

Still other surgeons in the 1950s and 1960s were reporting the results of local excision of breast tumors followed by breast irradiation. While failing to clearly determine the merits of that regimen they did demonstrate that patients could survive for many years free of disease following such treatment. More recent reports continue to attest to that fact. It is difficult to determine the rationale which was employed by the early advocates of local tumor excision. What seems most certain is that there was no clear biologic principle which directed their approach. In many instances, the operation was performed simply because patients refused to have a recommended radical mastectomy.

Despite the fact that clinical efforts between 1950 and 1970 failed to determine with certainty the relative merits of the various methods for the local-regional treatment of breast cancer, failed to evolve new biologic principles, and failed to test the halstedian hypothesis (allowing it to remain the paradigm for breast cancer management), operations of lesser extent were accepted in clinical practice and in some places were considered "standard." As has been said, the retreat from radical mastectomy was more the result of frustration with its inability to fulfill the tenets of the hypothesis than the result of new principles attracting attention. Analysis of the state of surgery for other solid tumors during this period of history would be parallel to that for breast cancer. Operations for other tumors continued to embody halstedian principles. Any variation in extent of operation was more likely related to the technical proficiency of the surgeon involved than to conceptual differences.

Laboratory Challenges of the Halstedian Hypothesis

During the time that anecdotal clinical information was accumulating, a series of laboratory and clinical investigations directed toward obtaining a better comprehension of the biology of metastases was being conducted by ourselves and others. We were entirely involved with experiment-driven research, fact findings, and our efforts were not stimulated by any convictions or hypotheses. These studies influenced our thinking relative to metastatic mechanisms. They revealed that the blood and lymphatic vascular systems are so interrelated that it is impractical to consider them as independent routes of tumor cell dissemination. Also, the residence of a vast majority of tumor cells gaining access to an organ via the bloodstream was transient. As a consequence, we concluded that patterns of tumor spread are not solely dictated by anatomic considerations but are influenced by intrinsic factors in tumor cells and in the organs to which they gain access. The thesis was accepted that there is no orderly pattern of tumor cell dissemination that could be based on mechanical and temporal factors.

Results from another series of experiments led to the conclusion that regional lymph nodes are not, as Virchow proposed, effective barriers to tumor spread. Lymph nodes trap rbcs, but tumor cells pass through nodes into efferent lymph. Tumor cells can gain access to the blood vascular system by lymphatic-venous communications in nodes. Additional studies indicated the biologic importance of regional nodes, which were found to have a role in both initiation and maintenance of tumor immunity. It was demonstrated that regional node cells are capable of destroying tumor cells; therefore, the presence of negative nodes may be a result of such a circumstance and because tumor cells traverse nodes, rather than a result of removal of a tumor prior to its dissemination. Regional lymph nodes from patients continued to possess immunologic capabilities despite the presence of growing tumors. Those qualities vary in nodes within a patient and between patients. The findings indicated that biologic rather than anatomic factors may be the reason that certain nodes contain metastases and others do not. Recent laboratory evidence indicates that regional node cells are instigators of a cascade of events giving rise to cytotoxic effector cells of both the lymphoid and myeloid series. Thus, we concluded that to consider such nodes merely as mechanical receptors for tumor cells and way stations for tumor dissemination is an anachronism.

Studies begun in 1958 indicated that host factors are important in the development of metastases and that a tumor is not autonomous from its host as was believed. Dormant tumor cells were demonstrated, and it was shown that perturbation of a host by a variety of means could produce lethal metastases from those cells. Our findings led us to consider that local recurrences following operation were apt to be the result of systemically disseminated cells lodging and growing at a site of trauma rather than of inadequate surgical technic. We proposed that a tumor is a systemic disease, probably from its inception. That premise never implied that all patients would at some time develop overt metastases, nor did it imply that only patients with metastases represent the population with disseminated disease.

In the 1960s the thesis was formulated that the regional node is an indicator of host-tumor relations. The lymph node that contains tumor cells reflects an interrelation between host and tumor that permits the development of metastases rather than reflecting a situation where the lymph node is an *instigator* of distant disease.

Concurrent with the laboratory studies, a series of clinical trials provided new information which raised questions regarding concepts upon which treatment was based. Recurrence and survival were found to be independent of the number of axillary nodes removed and examined, and tumor location failed to influence prognosis.

Formulation and Testing of an Alternative Hypothesis

Regardless of whether the results of all of the laboratory studies and clinical observations were interpreted correctly, whether they were due to the methodologies and models employed rather than to biologic circumstances, whether they could or could not be confirmed in every setting and in every detail, or whether they were obtained from simplistic experiments which did not really provide positive proof for any of our assumptions, they all had the same characteristic: they did not conform to the concepts which provided the principles for the halstedian hypothesis. They provided a matrix within which an alternative thesis could be formulated. That hypothesis, synthesized in 1968, is biologic in concept, rather than anatomic and mechanistic. Its components are completely antithetic to those in the hypothesis attributed to Halsted (table 1).

For a competitive theory to attract most of the next generation of practitioners, it would require confirmation. The opportunity to test the principles upon which the alternative hypothesis was based became available. In August 1971, a clinical trial was begun to confirm or deny the halstedian principles of cancer surgery and simultaneously to test the validity of the alternative hypothesis. The results of that trial indicate that in patients without clinical evidence of node involvement (40% of whom had histologically positive nodes), three distinctly different treatment regimens (radical mastectomy, total [simple] mastectomy with local-regional irradiation, or total mastectomy and removal of nodes that later became clinically positive) yielded no significant difference in overall treatment failure, distant metastases, or survival after a mean follow-up of 7 years (3). In patients with clinical evidence of node involvement, there was no significant difference between the group treated by radical mastectomy and the group treated by total mastectomy and local-regional irradiation.

The similarity in findings in patients with clinically negative nodes was remarkable, considering that ≅ 40% of women subjected to total mastectomy alone had positive nodes that were neither removed nor treated. Those nodes could have been expected to serve as a source of further tumor dissemination resulting in an increase in distant treatment failure and mortality. That such was not so provides justification for challenging temporal considerations in metastatic development and tends to indicate that even if metastases do further metastasize, the consequences, at least in this setting, are clinically irrelevant. Just as leaving nodes unremoved was not deleterious, their removal did not adversely affect prognosis, disproving the concept that leaving behind regional nodes may provide an immunologic advantage. When considered overall, the findings refute, in the human, the anatomic and mechanical precepts which have dictated thinking relative to metastases and have influenced surgical therapy. They lend support and confirmation to the validity of the various components of the alternative hypothesis. Unfortunately, the results of that study (and others to be described), which confirm the alternative hypothesis, are interpreted by most clinicians as providing justification for doing a lesser procedure and having no other meaning. The nonclinician laboratory investigator of metastasis is apt to overlook the biologic relevance of such results or more likely to be totally unaware of them.

Other clinical trials carried out during the past decade using breast cancer as the model have provided support for the alternative hypothesis. Of particular note in that regard is the Cancer Research Campaign (King's College, Cambridge, England) trial in the United Kingdom employing women with clinical stage I or II carcinoma of the breast (7). Survival results after 10 years indicated no difference in patients who had a simple mastectomy without surgical attention to the axillary nodes and those

TABLE 1.—Two divergent hypotheses of tumor biology

Halstedian hypothesis	Alternative hypothesis
Tumors spread in an orderly defined manner based upon mechanical considerations	There is no orderly pattern of tumor cell dissemination
Tumor cells traverse lymphatics to lymph nodes by direct extension, supporting en bloc dissection	Tumors cells traverse lymphatics by embolization, challenging the merit of en bloc dissection
The positive lymph node is an indicator of tumor spread and is the instigator of disease	The positive lymph node is an indicator of a host-tumor relationship which permits development of metastases, rather than the instigator of distant disease
Regional lymph nodes are barriers to the passage of tumor cells	Regional lymph nodes are ineffective as barriers to tumor cell spread
Regional lymph nodes are of anatomic importance	Regional lymph nodes are of biologic importance
The bloodstream is of little significance as a route of tumor dissemination	The bloodstream is of considerable importance in tumor dissemination
A tumor is autonomous from its host	Complex host-tumor interrelationships affect every facet of the disease
Operable breast cancer is a local-regional disease	Operable breast cancer is a systemic disease
The extent and nuances of operation are the dominant factors influencing patient outcome	Variations in local-regional therapy are unlikely to substantially affect survival
No consideration is given to tumor multicentricity	Multicentric foci of tumor are not of necessity a precursor of clinically overt cancer

who had a simple mastectomy and regional node radiotherapy. A review of all of the information obtained in the past decade or so from clinical trials has resulted in the conclusion that while there is evidence to confirm the alternative hypothesis, almost no substantive challenges to it are apparent.

The results of breast conservation recently reported from the National Cancer Institute of Milan (8) may also be interpreted as providing additional confirmation to the alternative hypothesis. They corroborate the premise contending that variations in local-regional therapy are unlikely to substantially affect survival. In that study, patients with tumors < 2 cm in diameter and with no palpable axillary nodes were treated, between 1973 and 1980, by Halsted radical mastectomy or "quadrantectomy" with axillary dissection and radiotherapy to the ipsilateral residual breast tissue. Actuarial curves have shown no difference between the two groups relative to outcome. Careful examination of that trial results in uncertainty regarding the rationale for the use of the less radical procedure when it began to be used in 1973. It is unlikely that the halstedian paradigm had been abandoned. The fact that the Halsted radical mastectomy was the standard against which the new operation was tested and that it was employed unchanged until 1978 when "the extent of surgery was slightly reduced with preservation of a large portion of the major pectoral muscle" suggests this. The fact that the quadrantectomy also continued to embody halstedian principles may be construed as evidence that those concepts still held sway; they were being modified but not abandoned. Attesting to this were: (*a*) the removal of a large amount of skin and breast tissue, the pectoral fascia, and the pectoralis minor muscle; (*b*) en bloc dissection in about 75% of the patients; and (*c*) the security contributed by the use of radiation therapy. While the results of this study provide a more credible transition from the halstedian paradigm to the one based on biologic principles than do anecdotal reports of experiences with lumpectomy, one cannot extrapolate from them and conclude what the outcome might have been if halstedian principles had been completely set aside.

The controversy regarding breast-conserving operations in women with mammary cancer is more than a clinical polemic. It has directed attention to the phenomenon of tumor multicentricity and to the realization that in the course of determining what constitutes optimal treatment for breast cancer there is the opportunity to obtain information which animal models and in vitro preparations are apt to provide with less certainty, if at all. There is a substantial incidence of women who have breast cancer with multifocal occult tumor in both breasts. Only rarely is there evidence of two or more overt cancers in the same breast; synchronous bilateral tumors are uncommon; and the incidence of an asynchronous primary tumor in the uninvolved breast fails by far to approach the incidence of occult lesions detected by random biopsy or at autopsy. As a result, there is justification for extending the alternative hypothesis so that it includes the premise that multicentric foci of tumor are not necessarily a precursor of clinically overt cancer (if they were, this would preclude operations that preserve the breast). Does the presence of a primary tumor inhibit the growth of other tumor foci and will its removal be followed by stimulation of their growth? Will there be an increased incidence of second tumors? Are occult tumor foci which are unremoved and which fail to become an overt lesion a persistent source of disseminated tumor cells which will increase the occurrence of distant disease and death? Answers to those simple fundamental questions should be obtained from a clinical trial now being conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP). At the completion of the NSABP study > 2000 patients will have been randomized so that they have received either an operation which removed the breast (total mastectomy and axillary node dissection) or one which conserved the breast (tumorectomy and axillary node dissection). One-half of those subjected to local tumor removal will have received breast irradiation with the intent of eradicating multicentric tumor foci and the other half will not have been treated with irradiation. Information provided by studying those patients should answer the preceding questions. Findings from the study will also provide information which will confirm or reject the various tenets of the alternative hypothesis.

How new knowledge relative to tumor biology may be employed to redefine the basis for an operative intervention is exemplified by considering how such information could impact upon breast-conserving operations. Recent information regarding tumor heterogeneity, receptor content of tumors, and other biologic considerations must eventually be taken into account when considering local tumor excision. Increasing evidence indicates that tumor estrogen receptor (ER) and progesterone receptor content, number of axillary nodes, age, pathologic characteristics, and other variables influence the outcome of breast cancer. It would be important to know if patients with ER-positive tumors are better candidates for lumpectomy without irradiation than are those who are ER-negative. Perhaps irradiation is highly effective only in the ER-negative group. Tamoxifen following segmentectomy in ER-positive patients may preclude the need for irradiation, even if it is proven to be of benefit in a particular patient subset. These and other questions having biologic clinical importance remain to be answered, and the surgeon is in the position to do so.

It now seems appropriate to set aside the paradigm which is the offspring of the halstedian hypothesis and accept a new one which is in keeping with the tenets of the alternative hypothesis—a paradigm which is based upon biologic principles. A transition from one paradigm to another has taken place via scientific revolution, just as in the usual development pattern of mature science. The current paradigm will be replaced by another in due time if progress is made.

Since the halstedian hypothesis has, over the years, provided the rationale for the operative management of almost all solid tumors, it is appropriate to consider whether, in light of its replacement as the paradigm for breast cancer management, it should be supplanted as the guide for handling other tumors as well. Has credible information been obtained from studies evaluating the surgical therapy of tumors other than those of the breast which supports or rejects the alternative hypothesis or which permits formulation of another thesis? Perhaps information from a Surgical Oncology Research Workshop sponsored by the National Cancer Institute in September 1981 could answer that question (9). The purpose of that workshop was to focus on four major cancers (melanoma, breast cancer, colorectal cancer, and soft tissue sarcoma) and to define areas relating to those aspects of the operation and other regional therapy which would be worthy of further investigation by surgeons. Unfortunately, a review of the proceedings of that meeting failed to provide an answer. Instead, it produced a sense of déjà vu. The presentations posed questions and raised issues which were highly reminiscent of those which were being directed toward breast cancer a decade or two ago.

A major concern in the treatment of melanoma still relates to how regional lymph nodes should be managed. Does an immediate or a delayed dissection provide the same survival benefit? How extensive should a nodal dissection be? Is there a difference between a dissection in continuity or a discontinuous one? What is the best way for a pathologist to handle lymph nodes, eg, node clearing, serial sections of nodes, etc? What is "the role of lymph nodes, their surveillance, their value in staging, and their biological role, if any"? What are the merits of regional therapy?

Questions regarding the management of soft tissue sarcomas were equally reminiscent of those previously directed toward breast cancer. What is the extent of resection required for satisfactory local control? Can a better surgical staging system be devised so as to better select the appropriate operation? Does adjuvant therapy (irradiation and/or chemotherapy) improve local control? Will adjuvant therapy improve survival?

It was concluded from the discussion directed toward colorectal cancer that, at present, it is more important to implement trials of adjuvant therapy than to be concerned with those directed toward evaluating modifications of the operative treatment. It was considered that "the primary emphasis should be the standardization of the operative approach utilized in systemic trials designed to improve end results."

From the deliberations of that workshop it would seem that the biologic significance of the findings relative to breast cancer surgery has been slow to be appreciated and slow to be applied to other solid tumors. While it is probable that the information obtained from the study of breast tumor has relevance for the surgical management of other cancers, scientific method requires that a proper evaluation be carried out in each situation to determine whether the tenets of the halstedian or the alternative hypothesis are valid. Such testing is not taking place relative to those tumors (except breast cancer) discussed at that workshop, nor is it being carried out relative to other appropriate neoplasms. Tumors of the testis, for example, are considered to be cancers most benefited by regional node removal or irradiation. The operation employed for treatment is in all respects halstedian, and its worth has never been satisfactorily tested by a proper prospective evaluation. Anecdotal retrospective information has provided the basis for the surgical procedure employed. With the advent of effective chemotherapy for that tumor there is an opportunity to properly reassess the surgical issue. Thyroid cancer and other head and neck tumors also require re-evaluation of their surgical management in light of the alternative hypothesis. It would thus seem that the evolution of the current paradigm for breast cancer surgery has had virtually no impact on the operative management of other tumors. In light of confirmation of the alternative hypothesis using breast cancer as the test model, there is ample justification for a full-scale re-evaluation of the basis upon which the surgical management of other tumors exists. There is urgency to reconfirm, deny, or modify the concepts which serve as the basis for operations used to treat them.

Another concern of the workshop relates to the plea for standardization of operative procedures at a time when the appropriate technic for the operative removal of the tumors is not defined. It is, of course, highly desirable that in clinical trials testing systemic therapy there should be an attempt to standardize the operation so that the only difference in patient groups is the therapy being tested. That does not imply, however, that such a "standard" has been proven to be an optimal operation.

Cancer Surgery in Conjunction With Other Modalities

As combined-modality therapy, ie, adjunctive chemotherapy, immunotherapy, and/or hormonal therapy, becomes more effective, all treatment directed toward local and regional tumor control (surgery and irradiation) will require reappraisal. The possibility exists that a therapy effective against distant disease may be equally effective against minimal residual, local, and/or regional disease. Consequently, an operation which is less effective than another of greater scope may, with the use of effective systemic therapy, become equivalent.

Another issue which arises with the use of multimodality therapy relates to the timing of the operation and to the administration of systemic therapy. Current practice dictates that following diagnosis of a tumor, surgical intervention is delayed only long enough for the attainment of a proper preoperative evaluation. Postoperative adjunctive therapy is employed only after there is adequate wound healing and patient performance status has approached normal. Before long, these temporal relationships are apt to be modified as the result of several recently formulated biologic premises which provide justification for considering the use of perioperative chemotherapy, ie, therapy administered prior to, at the time of, or shortly after primary tumor removal. One premise relates to the observation that removal of a primary tumor has an effect on the growth kinetics of metastases. Our own laboratory studies have demonstrated that within 24 hours following removal of a tumor there is an increase in the labeling index (LI) of a distant tumor focus which persists from 7 to 10 days (10). There is also a decrease in tumor doubling time and a measurable increase in tumor size which becomes apparent about 1 week following tumor removal. The tumor growth is probably a result of the conversion of noncycling cells in G_0 phase into proliferating cells, which should be more vulnerable to cytostatic agents. The rapidity of the onset of the kinetic changes and their relatively short duration provide a suitable rationale for the use of chemotherapy as soon as possible following tumor removal. In our investigations using an animal model, chemotherapy had a more favorable effect when given on the day of tumor removal, when the LI of metastases were at a peak, than 3 days later, when the LI had returned to the preoperative level (11). The greatest benefit occurred when the chemotherapy was given *prior* to operation. Use at that time completely prevented the increase in LI, more effectively suppressed tumor growth, and prolonged survival to a greater extent than was noted under any other circumstance. Those findings suggest that for more effective control of metastases chemotherapy had best be employed before or at the time of primary tumor removal. Aside from the therapeutic ramifications of the findings, their biologic impact requires assessment. Elucidation of the mechanism whereby removal of a primary tumor exerts its effect on metastases is worthy of investigation. What mediates such a phenomenon and what characterizes the cells that respond to the stimulus? It hardly needs pointing out that the kinetic changes observed by us and by others in animal models provide no assurance that a similar phenomenon takes place in humans following the removal of all or even some primary tumors of the same or different types. Moreover, there is no assurance that the temporal pattern of the kinetic changes in the animal and in the patient (should they occur) are similar.

Another justification for perioperative therapy is based upon the recent contention by Goldie and Coldman (12) that as a tumor cell population increases there is an ever-expanding number of drug-resistant phenotypic variants which become more difficult to eradicate. In that concept, "every minute counts." Consequently, combinations of non-cross-resistant drugs should be administered when a tumor population contains as few cells as possible. The Goldie-Coldman somatic mutation theory seems to provide an alternative and independent explanation to that evoking cell kinetic principles as the basis for drug resistance. The two are not, however, mutually exclusive. With the growth of a tumor, not only are the absolute numbers of resistant cells increased but so is the percentage of resistant cells in the total cell population. The latter is presumed to occur because resistant phenotypes not only multiply as a result of their own intrinsic growth rates but also as a consequence of the addition of new mutations from the pool of nonresistant (sensitive) cells. Following tumor removal, as a consequence of the enhanced proliferation of cells, it becomes more likely that the number of resistant phenotypes will increase in the metastatic population. Thus, appropriate perioperative therapy should not only destroy cells made more sensitive by their kinetic alteration but at the same time by preventing cell proliferation should prevent an increase of resistant cells.

A third justification for the use of perioperative therapy relates to repeated observations that surgical manipulation results in "showers" of circulating tumor cells (13,14). It has been reported that the presence of such cells is *not* related to patient outcome (15). Examination of the studies providing evidence for such a conclusion, however, reveals that they were "no contests" and could not have determined what the consequences of finding circulating cells might be. Insufficient patients with too short a follow-up time were inappropriately analyzed. Most important, outcome was related to the presence or absence of circulating tumor cells without regard for other variables which could have influenced prognosis.

Since perioperative therapy is apt to neutralize only events which are transient, ie, cells disseminated and kinetic alteration occurring during the perioperative period, there may be a question as to how much more beneficial such treatment will be than that which is conventionally given a few weeks to a month following operation. Is that "lead time" apt to alter significantly an outcome which is more likely determined by events prior to operation?

Despite such speculation, a trial of perioperative therapy is likely to provide more definitive biologic information relative to human neoplasms than will additional in vitro and in vivo models. If it is revealed from a study that has truly been a contest (a proper evaluation) that there is no benefit to the use of perioperative therapy, it may be concluded that the biologic consequences of primary tumor removal or of circulating tumor cells released at the time of operation are not likely to be of clinical significance and that further research in that aspect of metastasis should be diminished or redirected. Current biologic concepts will then need to be modified or abandoned.

Concept of a Surgical Oncologist

The current role of the surgeon in the management of cancer, as well as the definition of a surgical oncologist and how that individual might achieve parity with counterparts in medical and radiation oncology, is a frequent topic of discussion. From a limited and conventional perspective, the role of the surgeon is entirely the same as it has been since Halsted's time: to apply a particular intervention modality, a specific skill, as deemed appropriate to effect a therapeutic benefit. In that context, surgeons are cancer "therapists" and are in the same category as chemotherapists, who administer agents comprising the armamentarium of their intervention modality, or radiation therapists, who use the equipment of their speciality. For the most part, therapists are methodologically oriented and are concerned with the nuances of their particular resource rather than with concepts or scientific considerations which relate to their efforts. Surgical cancer therapists might, for example, ask how much of an axillary dissection is necessary rather than what might be the biologic basis for doing that which is being recommended. They too often view their counterparts in other disciplines as "competitive," a characteristic displayed by radiation therapists and chemotherapists as well.

In this era when so much emphasis is directed toward the multimodality treatment of cancer, while it is mandatory that surgeons be taught the skills and technical proficiencies which provide them with their unique capabilities, training programs must not in the process neglect to stress the biologic basis of cancer management. For surgical therapists, radiation therapists, chemotherapists, and immunotherapists to be equal participants in determining treatment, they must all have an equivalent knowledge of the disease they are managing. Only when they have the same basic knowledge of oncology and differ only in the modality which they employ is there a true multimodality approach to cancer therapy.

Oncology is defined as "the sum of knowledge concerning tumors; the study of tumors." As a consequence, an oncologist, as perceived by me, is an individual who has a current understanding of tumor biology with the capability of selecting from the "oncologic concept pool" that which can be utilized in clinical investigations and/or applied to the formulation of better therapeutic strategies directed toward the patient (target) (fig 1). An oncologist should also possess the potential for generating new information via laboratory and clinical research which can add to the information pool.

By the very nature of the physical and temporal rigors as well as intellectual distractions, present programs of surgical training obtund the development of surgeons who might be in a position to advance the science of oncology. They are, for the most part, taught to think in terms of process and methodology (technic) rather than in terms of concept and biology. It is vital that those in charge of training programs realize that each discipline affords a different pathway for becoming involved in the study of cancer. Surgery can and must provide such a route for a modest number of individuals who are interested in becoming and/or have the capabilities to become oncologists. True surgical oncologists will utilize information from the pool of information to answer questions which are apt to be different from those asked by individuals who have become oncologists by way of other disciplines. Experience encountered by surgeons will result in the asking of questions which are related to their particular orientation to the disease. Professionals in other disciplines will do the same. The medical oncologist may, for example, be drawn toward pharmacologic and kinetic questioning and the radiation oncologist to questions related to radiation biology. The trained surgical oncologist-biologist perceives areas of investigation which can affect the target and which are not discernible to those in other disciplines. Nonclinical investigators (PhD) are at a

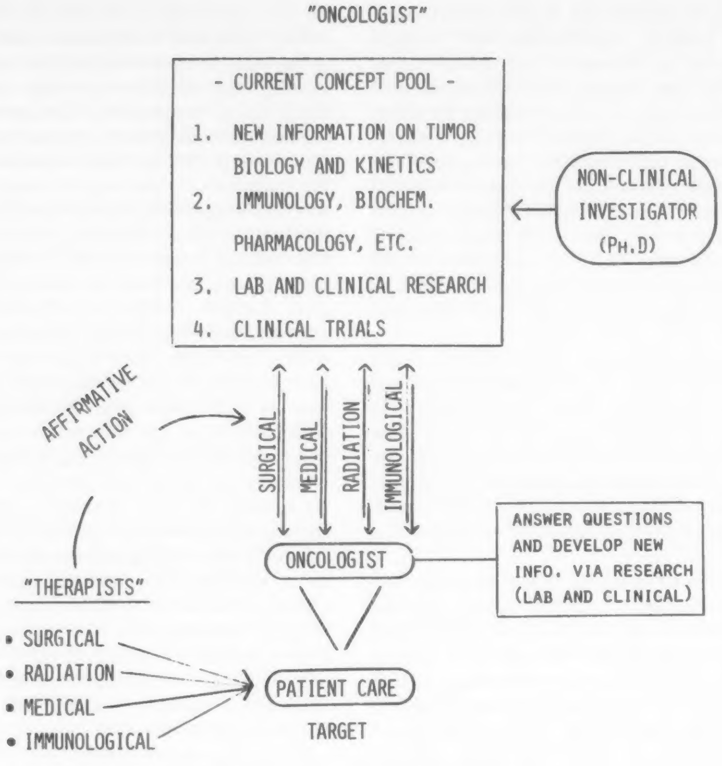

"ONCOLOGIST"
- CURRENT CONCEPT POOL -
1. NEW INFORMATION ON TUMOR BIOLOGY AND KINETICS
2. IMMUNOLOGY, BIOCHEM. PHARMACOLOGY, ETC.
3. LAB AND CLINICAL RESEARCH
4. CLINICAL TRIALS
NON-CLINICAL INVESTIGATOR (PH.D)
AFFIRMATIVE ACTION
SURGICAL
MEDICAL
RADIATION
IMMUNOLOGICAL
ONCOLOGIST
ANSWER QUESTIONS AND DEVELOP NEW INFO. VIA RESEARCH (LAB AND CLINICAL)
"THERAPISTS"
• SURGICAL
• RADIATION
• MEDICAL
• IMMUNOLOGICAL
PATIENT CARE
TARGET

FIGURE 1.—Development of the surgical oncologist.

decided disadvantage in that, while they have the capacity of adding to the data pool, they are unable to direct information from it to the target. Any affirmative-action program for surgical oncology must be entirely directed toward making it possible for a subset of surgeons to expand beyond their methodologic orientation so that they become scientists who can introduce a different perspective into the study of cancer. The principal aim of that effort must be to capture the intellect of those surgeons who are capable of making a contribution to oncology which extends well beyond the technical aspects of their discipline. By such a program surgical therapists become oncologists (fig 1).

REFERENCES

1. FISHER B. The changing role of surgery in the treatment of cancer. *In* Cancer: A Comprehensive Treatise (Stoll BA, ed). New York, Plenum Publishing Corp, 1977, vol 6, chap 10, pp 401-421.
2. ______. A commentary on the role of the surgeon in primary breast cancer. Br Cancer Res Treat 1:17-26, 1981.
3. ______. Laboratory and clinical research in breast cancer—a personal adventure: the David A. Karnofsky Memorial Lecture. Cancer Res 40:3863-3874, 1980.
4. ______. The role of science in the evolution of breast cancer management. *In* Current Controversies in Breast Cancer. New York, Raven Press. In press.
5. ______. The interdependence of laboratory and clinical research in the study of metastases. Keynote Address presented at the 1983 Annual Symposium on Fundamental Cancer Research, M. D. Anderson Hospital and Tumor Institute, Houston, TX, Feb 28-Mar 3, 1983. In press.
6. CRILE G, JR. Simplified treatment of cancer of the breast; early results of a clinical study. Ann Surg 153:745-761, 1961.
7. Cancer Research Campaign Working Party. Cancer Research Campaign (King's/Cambridge) trial for early breast cancer—a detailed update at the tenth year. Lancet 1:55-60, 1980.
8. VERONESI U, SACCOZZI R, DELVECCHIO M, ET AL. Comparing radical mastectomy with quadrantectomy, axillary dissection and radiotherapy in patients with small cancers of the breast. N Engl J Med 305:6-11, 1981.
9. LAURENCE DW, and GHOSH BCL. Surgical Oncology Research Workshop, September 9, 1981. Division of Cancer Treatment, National Cancer Institute, NIH Publication No. 82-2444, Jan 1982.
10. GUNDUZ N, FISHER B, and SAFFER EA. Effect of surgical removal on the growth and kinetics of residual tumor. Cancer Res 39:3861-3865, 1979.
11. FISHER B, GUNDUZ N, and SAFFER EA. Influence of the interval between primary tumor removal and chemotherapy on kinetics and growth of metastases. Cancer Res 43:1488-1492, 1983.
12. GOLDIE JH, and COLDMAN AJ. A mathematic model for relating the

drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 63:1727-1733, 1979.

13. FISHER ER, and TURNBULL RB. Cytologic demonstration and significance of tumor cells in the mesenteric venous blood in patients with colorectal carcinoma. Surg Gynecol Obstet 100:102-108, 1955.
14. MOORE GE, SANBERG AA, and SCHULBARG JR. Clinical and experimental observations of the occurrence and fate of tumor cells in the bloodstream. Ann Surg 146:580-587, 1957.
15. SALSBURY AJ. The significance of the circulating cancer cell. Cancer Treat Rev 2:55-72, 1975.

Tumor Stem Cell Heterogeneity: Implications With Respect to Classification of Cancers by Chemotherapeutic Effect

Howard E. Skipper* and Frank M. Schabel, Jr[1,†]

Herein we have considered the types of tumor stem cell heterogeneity that might account for (*a*) fluctuating degrees of response to chemotherapy in similarly treated individuals bearing a particular neoplasm, and (*b*) classifications of cancers by chemotherapeutic effect. Fluctuating ratios of T/R to T/0 stem cells, as predicted by the mutation theory, will account for (*a*). In different circumstances at least three phenomena might account for (*b*): growth fraction differences, differences in T/R to T/0 stem cell ratios, or differences with respect to pharmacologic sanctuaries. T/R stem cells are primarily responsible for the failure of the best available chemotherapy to cure responsive, refractory, and very refractory experimental neoplasms. The data examined suggest that differences in the T/R to T/0 stem cell ratios in different types of cancer may account for their being classed as responsive, refractory, or very refractory. If this be true, what might underlie such differences? The most obvious possibilities are: higher mutation rates to a drug-resistant state in refractory cancers, or some sort of "natural selection" of diverse T/R stem cells in refractory cancers. [Cancer Treat Rep 68:43–61, 1984]

When we received an invitation to contribute to this anniversary issue of *Cancer Treatment Reports* we had just finished writing an informal report entitled "Some Thoughts on Tumor Heterogeneity and Tumor Stem Cell Heterogeneity." Throughout this long report an effort was made to focus on questions such as these:

Question A: Why is it so common to observe widely fluctuating degrees of response to chemotherapy in similarly treated individuals bearing similar burdens of a particular neoplastic disease?

Question B: What biologic phenomena underlie "classifications of cancer by chemotherapeutic effect"; eg, responsive, moderately refractory, or very refractory or, in Holland's terms, curable, subcurable, or precurable?

In an attempt to shed light on these questions which have puzzled experimentalists and clinicians for many years, extensive data were examined in the context of more specific questions.

Is the heterogeneity in clinical response of cancers to chemotherapy a reflection of tumor stem cell heterogeneity—somewhat as is depicted in figure 1?

What type of tumor stem cell heterogeneity is *primarily* responsible for differences in response in different circumstances: (*a*) variations in the relative proportions of stem cells in Compartment B versus Compartment A (the growth fraction) or (*b*) variations in the relative proportions of stem cells in Compartments R_1, $R_{1,2}$, and R_n versus Compartments A and B (the mutant drug-resistant fraction)? A reliable answer(s) to this question seems important because the biochemical reasons for drug resistance due to both variations mentioned are totally different. The same may be said regarding strategies required to minimize chemotherapeutic failures due to these phenomena.

In this paper an attempt will be made to recount in summary fashion the conclusions reached in the long informal report mentioned earlier; that is, deductions reached after examining many types of data for compatibility and lack of compatibility.

As different types of experimental and clinical data are considered, we will try not to forget the difficulties in quantitative measurement of the tumor stem cell kill by chemotherapy (total and by stem cell type), particularly in human cancers; the inverse relationship between tumor burden and curability with chemotherapy, but with quantitative differences between cancers; and the direct relationship between tumor burden and age and the probability of the presence of single-, double-, and multidrug-resistant tumor stem cells, but again with quantitative differences between cancers.

---

[1] Southern Research Institute, Birmingham, AL.
†Deceased.

**Reprint requests to:* Howard E. Skipper, PhD, Southern Research Institute, PO Box 55305, Birmingham, AL 35255-5305.

BACKGROUND

Recent Clinical Analyses

In recent lectures Holland has said things that needed to be said and offered "names" and characteristics of "solid" tumors, a classification of human cancers by chemotherapeutic effect, and some reasons for chemotherapeutic failures (1,2). In table 1 we tried to summarize the essence of Holland's tabulations. With some quantitative changes regarding burden and growth rates, what he wrote about human cancers would apply just about as well for a variety of experimental cancers we have studied.

DeVita (3) in his James Ewing lecture emphasized the relationship between tumor mass and resistance to chemotherapy. He noted that tumor mass negatively affects the outcome of cancer chemotherapy in a manner quite different from the way in which it does surgery or radiotherapy.

> "Cancer chemotherapy fails because cells develop resistance to anticancer drugs. Conceptually, there are two types of resistance, both of which are mass related: temporary resistance (due to pharmacologic sanctuaries or altered cell kinetics) or permanent resistance (mutant lines developing specific and permanent resistance to one or more cancer drugs). Based on the somatic mutation theory, it now appears that resistant mutants arise spontaneously early in the natural history of cancers, and the likelihood of a resistant line developing appears closely related to cell number, such that one or more resistant lines are likely to present *before* most human malignancies become clinically evident. The development of permanent resistance more precisely accounts for the invariable inverse relationship between cell number and curability by drugs and the greater effectiveness of combination chemotherapy over single agents."

We have quoted rather extensively from the summary of DeVita's lecture because his remarks are particularly pertinent to *Questions A* and *B*, posed in the introduction, and to the thoughts on tumor stem cell heterogeneity in relation to tumor responsiveness offered in figure 1.

Mathematical and Experimental Models and Their Predictions[2]

The simple mathematical model of Lloyd has been of great help in more quantitative interpretations of experimental chemotherapy trials (4).

The net tumor stem cell decrease or increase during treatment

$$\text{(logs)} = \quad [K_s \times n] \quad - \quad [\frac{\text{Log } 2}{DT} \quad (n - 1)(I)]$$

Where K_s = the sensitive tumor cell kill per dose or course (logs)

n = the number of doses or courses

DT = doubling time in near log phase (days)

$\frac{\text{Log } 2}{DT}$ = growth rate in fractions of a log per day

I = the interval between doses or courses.

This relationship does not take into account the presence of permanently drug-resistant neoplastic stem cells prior to initiation of treatment or emerging during treatment. It is now abundantly clear that this type of drug resistance is a major cause of chemotherapy failure in experimental cancers that are responsive, relatively refractory, or very refractory to chemotherapy. Curve *ad* in figure 2 illustrates what might be expected with very effective chemotherapy in the absence of drug-resistant tumor cells (T/R stem cells). Unfortunately, in relatively advanced experimental and human cancers, single drug treatment and combination chemotherapy all too often provide end-results that are consistent with the time-action curve *ac* in figure 2.

At this point it seems apparent that resting T/0 stem cells are refractory to many types of antimetabolites, mitotic inhibitors, and possibly (in smaller degree) to alkylating agents and DNA binders if some such cells achieve "repair" prior to resumption of DNA replication and division. But this type of resistance will *not* account for the very common observation of tumor regression [minimum, partial response (PR), or complete response (CR)] followed by progression during continuing undiminished treatment with the same drug or combination of drugs as in curve *ac* figure 2 and in many conclusive experimental data.

In 1979 Goldie and Coldman (5) provided a mathematical model based on the somatic mutation theory of Luria and Delbruck (6) that has been of much help to us in interpretation of chemotherapeutic results and in future planning. This model incorporated the following assumptions.

1. That cells which are stably resistant to a chemotherapeutic agent, at any specified dose, arise as a result of random mutations.
2. That such resistant cells have the same growth kinetics as the drug-sensitive tumor cells.
3. That essentially all tumor cells are capable of unlimited proliferation, ie, they are stem cells.

Their model, when integrated with the model of Lloyd in a computer program, made it possible to carry out

---

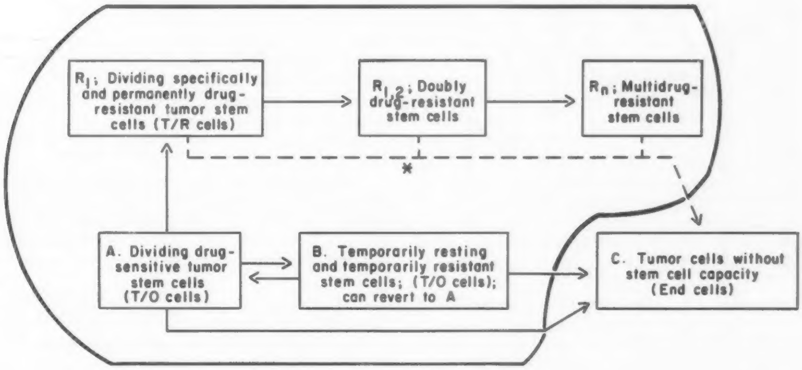
[2] Abbreviations used in this section: T/0 cells = drug-sensitive or potentially drug-sensitive tumor stem cells; temporarily resting T/0 cells are refractory to certain classes of drugs until they resume DNA replication and division (*see* fig 1). T/R cells = specifically and permanently drug-resistant tumor stem cells. These may be resistant to only one drug or drug class, or to two or more drugs or drug classes. Examples: (*a*) L1210/ara-C = ara-C-resistant L1210 leukemia stem cells. (*b*) L1210/CPA/ara-C = L1210 leukemia stem cells that are resistant to both cyclophosphamide and ara-C. If the burden of L1210/CPA cells is relatively high they will contain small numbers of L1210/CPA/ara-C cells that are selected in vivo by ara-C treatment. K_s = sensitive tumor stem cell kill per dose or short course of a drug (in logs or fractions of a log).

TABLE 1.—Classification of human cancers by chemotherapeutic effect (1980); *from* ref 1*

	Curable	Subcurable	Precurable
Curability:	⩾ 50%	< 50%	Uncommon
Morphology:	Undifferentiated	Partially differentiated	Partially differentiated
Growth rate:	Relatively rapid	Less rapid	Less rapid
Growth fraction†:	Relatively high	Relatively low	Low
Influence of size:	Moderately independent	Critical	Critical
	Choriocarcinoma Wilm's tumor Pre-T cell ALL Burkitt's tumor Hodgkin's disease Acute promyelocytic leukemia Diffuse histiocytic lymphoma Embryonal carcinoma of the testes	T-cell ALL Acute myelocytic leukemia Small cell carcinoma of lung Squamous cell carcinoma of upper aerodigestive tract Adenocarcinoma of ovary Adenocarcinoma of breast Osteosarcoma Ewing's sarcoma Embryonal rhabdomyosarcoma	Lung carcinomas other than small cell Carcinomas of esophagus, stomach, pancreas, liver, biliary tract, and colon Carcinoma of endometrium, cervix Carcinoma of kidney, bladder, and prostate Carcinoma of thyroid Gliomas Melanoma Carcinoids CML CLL Multiple myeloma

*Holland emphasized the importance of tumor burden with respect to the potential for cure by chemotherapy, eg, the importance of combination modality treatment. Limitations associated with biochemical resistance also were considered.

†Presumptions generally consistent with growth rates in near exponential phase; cytokinetic data not available for all types listed.


R_1; Dividing specifically and permanently drug-resistant tumor stem cells (T/R cells)
$R_{1,2}$; Doubly drug-resistant stem cells
R_n; Multidrug-resistant stem cells
*
A. Dividing drug-sensitive tumor stem cells (T/O cells)
B. Temporarily resting and temporarily resistant stem cells; (T/O cells); can revert to A
C. Tumor cells without stem cell capacity (End cells)

Tumor Stem Cell Compartments

*Resistant phenotypes also may go into and out of resting phase but they remain T/R cells.

FIGURE 1.—Some thoughts on tumor stem cell heterogeneity. (*a*) This representation excludes the normal cells present in tumors, eg, large proportions in Hodgkin's disease masses. (*b*) In the stem cell compartments, Compartment B is apt to be larger in slowly growing tumors than in rapidly growing tumors. In addition, the T/R to T/0 ratio is likely to be higher in slowly growing than in rapidly growing neoplasms of the same size. It seems probable that this ratio is rarely as high as 1.0 at the beginning of treatment even in very refractory tumors. After selection to the point where this ratio is > 1.0, tumor progression will be observed during continuing treatment with the same drug(s). (*c*) Compartment C (nonstem cells) is apt to be the largest compartment in partially differentiated, slowly growing tumors. (*d*) In prospective design of treatment regimens it is important to attempt to deduce which of the tumor stem cell types is apt to be limiting.

simulations of hundreds of past experimental chemotherapeutic trials and to strengthen and extend the interpretations of most of them. Such simulations were especially helpful in establishing criteria for optimum delivery of non-cross-resistant combinations of drugs in animals bearing drug-sensitive and relatively refractory cancers.

More recently Goldie and Coldman (7) offered a second model which is a modification of their original minimal model. For good reasons they suggest that the second model more closely approximates real systems and may provide insight into the processes that result in clinical tumors being relatively refractory or very refractory to chemotherapy. Briefly, they removed the assumption that essentially all tumor cells have stem cell capacity and examined (mathematically) the effect of a stem cell compartment in which a proportion of cells randomly lose the capacity for unlimited growth, eg, as in Compartment C in figure 1. It was noted that comparatively large rates of egress of tumor stem cells to a nonstem cell compartment (eg, differentiation) lead to larger fractions of the stem cell compartment being resistant (T/R cells). Or, in other words, "long lineages lead to comparatively greater degrees of resistance." "Furthermore, doubly resistant cells will accumulate at a greater rate than can be offset by their likelihood of differentiating out of the stem cell compartment of the tumor."

This model, which tends toward better compatibility with clinical experience with refractory cancers, is the first we have seen which suggests that differentiation and cell death not only contribute to slower tumor growth but also contribute to a significant increase in the proportion of single-, double-, and multi-drug-resistant stem cells at a given tumor size. Thus, it is not unreasonable to postulate that the general relationship between degree of differentiation, growth rate, tumor size, and age versus responsiveness to chemotherapy may be a reflection of the rate of "natural selection" of permanently drug-resistant tumor stem cells (T/R$_1$, T/R$_{1,2}$, and T/R$_n$ cells). In this context, temporarily resting and temporarily resistant tumor stem cells that can and do revert to a drug-sensitive state may be a major limitation only with respect to the method of delivery of drugs, especially antimetabolites like cytarabine (ara-C). Such tentative con-

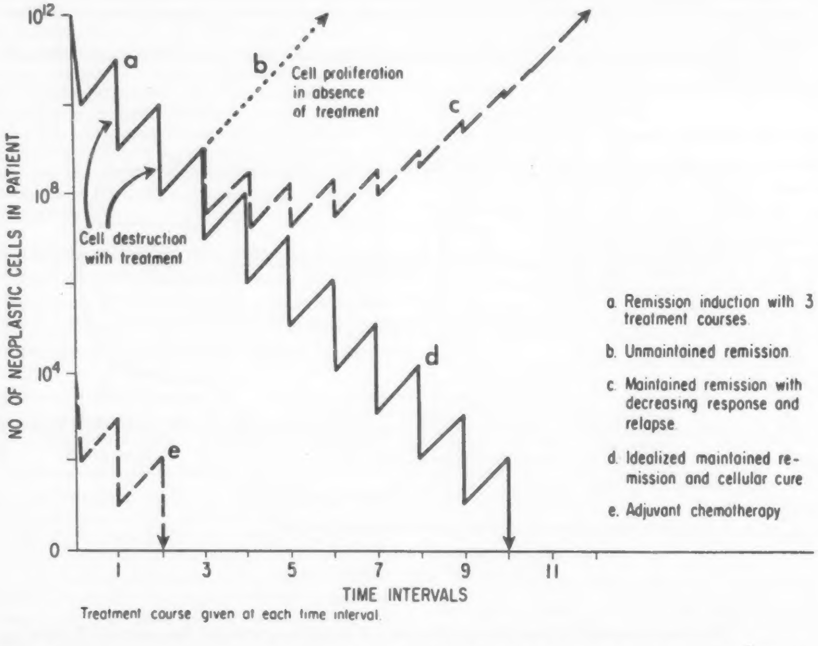


FIGURE 2.—Idealized time-action curves (from Blum, Frei, and Holland, *Cancer Medicine*, 2nd ed, 1982). Curve *ab*: cessation of treatment after 3 courses of chemotherapy. A 2nd response with the same treatment might or might not be possible depending on the T/R to T/0 stem cell ratio at the cessation of treatment and at relapse. Curve *ac*: a classical illustration of response followed by tumor progression during continuing undiminished treatment with the same drug or drugs. The nadir will be reached when the surviving tumor stem cell burden is comprised of about 50% T/R cells. Variations in the original T/R to T/0 ratio will influence the degree of response. Curve *ad*: What might be observed in the absence of T/R cells at the beginning of treatment or emerging during treatment, ie, T/R cells that were resistant to the drug(s) employed. Curve *e*: cure of relatively small burdens of tumor stem cells after surgery or radiotherapy; no T/R cells that were resistant to the drug or drugs employed.

cepts seem quite pertinent to *Question B*.

We also found a recent paper by Mackillop et al (8), "A Stem Cell Model of Human Tumor Growth: Implications for Tumor Cell Clonogenic Assays," of much interest. These authors considered three tumor cell populations: stem cells, transitional cells (proliferating cells that do not give rise to stem cells), and end cells. They suggest that in some human cancers it is possible that only $\leqslant 0.1\%$ of the tumor cells are stem cells. (To us this seeks a likely situation in many solid animal and human cancers when they are moderately advanced.) They make the following points regarding the relevance of their model to the outcome of cytotoxic therapy.

Cure of a tumor requires eradication of all cells with repopulation capacity; cure is a function only of irreversible stem cell inactivation or loss. Likewise, the rate of tumor regrowth is related to the number of stem cells surviving subcurative therapy.

Complete remission is the best predictive parameter of improved survival because this response is likely to relate to loss of tumor stem cell function. However, short-term reduction in tumor volume is a less reliable parameter because this might be a reflection of lysis of cells in the end-stage or cell death in the transitional cell population.

Table 2 lists model predictions that may serve as a checklist with respect to data and theory that might or might not be pertinent to *Questions A* and *B*. Most particularly, we would like to emphasize Items 16, 17, and 18 in table 2. These refer to the implications of PR and CR that are followed by tumor progression during continuing undiminished treatment with the same drug(s).

DRUG-SENSITIVE EXPERIMENTAL NEOPLASMS

Murine Leukemias

Some investigators who have not worked with murine leukemias seem not to like them for a variety of reasons, both real and imagined. But it is not difficult to document that these systems have provided biologic and therapeutic response information that has been helpful in developing more effective treatment for a variety of disseminated human cancers. Of course, transplantable leukemias are not good kinetic or therapeutic response models for, say, human melanoma (or mouse melanoma). Neither are human acute leukemias. Essentially all of the cytotoxic drugs that are useful in treating human cancers are effective in one or another of the experimental leukemias. No one knows whether these systems might miss an agent(s) that would be very effective against some chemotherapeutically refractory human or experimental neoplasm(s).

One characteristic of transplantable murine leukemias that is different from most solid tumors is the high fraction of neoplastic cells which have stem cell capacity, eg, approximately $\geqslant 50\%$ versus a lower fraction in most ad-

TABLE 2.—Observations and predictions based on mathematical and experimental models*

1. Small tumor burden more curable than large
2. Variable response for equivalent burden
3. Curability lost over small (≅ 2 log) increase
4. Combination superior to single agent
5. Drugs and hormones superior to either alone
6. "Front end loaded" therapy superior to maintenance
7. Alternating non-cross-resistant superior to sequential

……

8. Intensive chemotherapy superior to lower doses over longer periods
9. Simultaneous combinations superior to alternating; alternating superior to sequential—depending on dose levels, K_s sums and ratios, schedule, and limitations due to toxicity
10. Surgery + chemotherapy superior to either in disseminated disease (related to point 1)
11. Curability limited by CNS disease
12. Tumor stem cell populations with specific and permanent resistance to essentially all classes of anticancer drugs have been selected in vivo (including double and multi-drug-resistant phenotypes). Often this is observed without repeated treated passage
13. There is a direct relationship between neoplastic cell burden and the probability of the presence of specifically drug-resistant tumor stem cells (related to points 1, 3, 5, 6, 8, 10 and others)
14. Rate of selection of drug-resistant populations directly related to K_s and the original T/R to T/0 stem cell ratio
15. Minimum responses, PR, and CR followed by tumor progression during continuing undiminished treatment with single drugs and combinations is a common observation at the experimental level as it is at the clinical level (*see* curve *ac* in fig 2)
16. PR followed by progression during continuing R_x imply that by the time the T/0 stem cells have been reduced to unmeasurable dimensions the T/R cells already have increased to measurable dimensions
17. CR followed by relapse and progression during continuing R_x imply that at the time of the CR the T/0 cells had been reduced to unmeasurable dimensions and the T/R cells had not yet increased to measurable dimensions
18. CR imply a greater reduction in total burden than PR but not necessarily a greater reduction in the T/0 stem cells
19. Effective but noncurative chemotherapy consistently increases the survival time variance over controls; ineffective chemotherapy does not. In experimental neoplasms all that is necessary to cover up drug-induced increases in survival time variance is to add a constant number of drug-resistant cells to the inocula (eg, 0.1%)
20. Cure by single drugs is unequivocally limited by the presence or emergence of T/R stem cells. Tumor progression during combination chemotherapy may occur when the preponderance of the surviving tumor cells are resistant to only one drug in the combination
21. Temporarily resting and temporarily refractory T/0 cells, that can and do revert to cycle, seem to be a major limitation only with respect to the method of drug delivery, especially antimetabolites like ara C

*Points 1-7 listed are *from* ref 9; remainder are from informal reports from Southern Research Institute.

TABLE 3.—Some observations in murine leukemias and their implications

Leukemia	Observation*	Implications	Some references
1. AK	In vivo sterilization of about 10^8 leukemia cells by 2–8 times the LD_{50} of alkylating agents but not by > 100 times the LD_{50} of antimetabolites	These early studies pointed to some remarkable differences between alkylating agents and antimetabolites. Later it was observed that resting T/0 cells are not nearly as refractory to alkylating agents as to antimetabolites. In an absolute sense, alkylating agent-resistant T/R cells are not nearly as resistant to the alkylating agent that selected them as antimetabolite-resistant T/R cells to the antimetabolite that selected them, eg, < 5-fold vs > 1000-fold, respectively	Burchenal et al (10)
2. L1210	Resting leukemia stem cells are very refractory to antimetabolites and mitotic inhibitors but not to alkylating agents or DNA binders	Antimetabolites and mitotic inhibitors do not kill stem cells that are not replicating their DNA and dividing during exposure. Alkylating agents or DNA binders kill dividing and resting leukemia stem cells alike if the latter attempt DNA replication and division prior to repair (excepting specifically resistant T/R cells)	Schabel et al (11)
3. L1210, P388, AK, and others	If the leukemia cell burden is too high to cure with single drug treatment, and repetitive dose treatment is employed, specifically and permanently resistant stem cells (T/R cells) are selected by essentially all classes of anticancer drugs. This usually can be demonstrated in the original host	These almost monotonously consistent observations imply (*a*) a direct relationship between the leukemia cell burden and the probability of the presence of T/R stem cells, and (*b*) that selection and overgrowth of T/R stem cells is a major cause of chemotherapeutic failure	Burchenal et al (12), Law (13), Hutchison and Schmid (14), Skipper (15), and Brockman (16)
	Combinations of non-cross-resistant drugs are consistently more effective than single drug teatment if optimally delivered, ie, with attention to dose levels, K_s (total and ratios), intervals between treatment, and toxicity	Combinations are more effective than single drug treatment because they delay or prevent overgrowth of T/R stem cells. When combinations fail during continuing undiminished treatment, the surviving leukemia stem cells are resistant to one or more but not necessarily to all of the drugs in the combination. Singly drug-resistant cells mutate to doubly resistant cells and these may in turn mutate to multidrug-resistant phenotypes (T/R_n stem cells)	Schmid et al (17), Skipper (4), and Schabel et al (18)
4. L1210 and others	A given dose of a given drug will kill the same fraction, not the same numbers of different-sized tumor cell populations—as long as their growth fraction and phenotypic heterogeneity are the same	Compatible with first-order or pseudo first-order chemical reaction rates and early postulates of the mechanisms of in vitro cell killing of micro-organisms by disinfectants	Skipper et al (19)
	There is an inverse relationship between the tumor stem cell burden and curability with chemotherapy	Consistent with (*a*) the direct relationship between the tumor stem cell burden and the probability of the presence of T/R cells, (*b*) the inverse relationship between tumor burden and growth fraction, and (*c*) the direct relationship between tumor burden and the probability of tumor cells in pharmacologic sanctuaries	
	Antimetabolites and Mitotic Inhibitors		
5. AK, L1210, and others	(*a*) Dose-response curves (short-term treatment): almost exponential over lower dose range and then breaking to near horizontal at higher doses (excepting 5-FU). Dose-response curves for marrow stem cells not as steep as for leukemia stem cells	(*a*) If the leukemia cell burden is high and the duration of the exposure is ≤ 24 hrs, the surviving stem cells are likely to be resting T/0 cells and somewhat lower Nos. of T/R cells; must be passed and tested to determine which are preponderant. (We presume that resting marrow stem cells	Bruce et al (20) and van Putten (21)

		are responsible for the horizontal portions of their dose-response curves. Drug-resistant marrow stem cells have not been observed.)	
	(*b*) Time-action curves (long-term treatment) at a given dose level: if the burden is too high to cure, T/R cells overgrow and kill the host (extensively documented)	(*b*) It is very common to observe results consistent with curve *ac* in fig 2 if repetitive doses are continued until host death rather than for $\leqslant$ 24 hrs as is used in dose-response curves, ie, failure due to T/R cells	
	Alkylating Agents and DNA Binders		
	(*a*) Dose-response curves (short-term treatment): exponential almost to the MTD with a slight decrease in slope at higher doses; marrow stem cell curves not as steep as leukemia stem cells	(*a*) Surviving leukemia stem cells may be due to resting T/0 cells, T/R cells, or both	Bruce et al (22) and van Putten (21)
	(*b*) Time-action curves (long-term treatment with a given dose level): if the burden is too high to cure, T/R cells may overgrow	(*b*) Leukemia stem cells that are resistant to repetitive dose levels employed overgrow during treatment. These T/R cells may respond to higher single doses of the same agent. (Supralethal doses of cyclophosphamide will kill 3-5 logs of L1210/CPA cells that are almost completely resistant to a single LD_{10} of CPA.)	Skipper (4)
6. L1210	High fluctuations in the response to MTX of animals bearing independent sublines of L1210 leukemia (all arising from small Nos. of the same population and repeatedly passed in untreated animals)	Compatible with a basic tenet of the somatic mutation theory	Law (23)
7. L1210 and others	Effective but noncurative chemotherapy consistently increases the survival time variance in individual animals (similarly staged and similarly treated); ineffective chemotherapy does not. (a consistent observation for > 20 yrs) All that is necessary to cover up drug-induced increases in survival time variance (the "naked variance") is to add to the inocula a constant No. of leukemia stem cells that are specifically resistant to the drug to be employed (eg, 0.1%)	Consistent with *6*. This is precisely what should be expected if we accept a basic tenet of the somatic mutation theory	Skipper (4)
8. First passage AK leukemia and spontaneous AK leukemia	First passage AK leukemia is just about as responsive and "curable" as L1210 or P388 leukemia, but when young animals are cured they eventually die of spontaneous leukemia 5 mos to 1 yr later. The host is bearing the Gross virus. Spontaneous AK leukemia appears about as responsive to a wide variety of drugs as 1st passage or long passage lines, but if "cured" the animals suffer reinduction	I have sometimes wondered whether some of the chemotherapeutic failures in human leukemias and lymphomas are a result of reinduction of the disease after eradication of the neoplastic stem cells	Schabel et al (24) and Skipper et al (25)

---

[*] Where studied the above observations carry over to solid tumor systems, albeit with quantitative differences.

vanced solid tumors. This has advantages from a technical standpoint (one's ability to stage accurately) but it makes the leukemias less like partially differentiated and refractory tumors. The high stem cell level in leukemias may also result in a lower proportion of T/R stem cells per unit cell number or mass than obtains in refractory solid tumors with larger nonstem cell compartments.

Because much has been written about the biologic nature and therapeutic response of murine leukemias, we will point to only a few observations made in these systems (*see* table 3).

The implications of these observations seem internally consistent (in a cross-disciplinary sense) and they also seem to shed light on *Question A*: namely, wide fluctuations in the degree of response to chemotherapy of similarly treated individuals bearing similar burdens of a particular neoplasm are precisely what should be expected if we accept a basic tenet of the somatic mutation theory (4).

The observations in table 3 also seem pertinent to a part of *Question B*. The type of neoplastic stem cells primarily responsible for the failure of chemotherapy to cure animals bearing relatively advanced leukemia is T/R stem cells, not resting T/0 stem cells. Murine leukemias are responsive to a wide variety of drugs.

Plasmacytoma (Pla-1) in the Hamster

In the 1960s Griswold et al (26) carried out extensive chemotherapy trials and ancillary studies using the Fortner plasmacytoma (Pla-1) in the hamster. In near exponential phase, the volume doubling time of this neoplasm is about 1 day, and the pulse thymidine index is about 10% compared to about 65% for L1210 leukemia. Pla-1 responds poorly to antimetabolites but is very responsive to alkylating agents. In many trials in which animals bearing 1-g Pla-1 tumors were treated repetitively with cyclophosphamide, high CR rates were observed with varying cure rates. However, in essentially all trials when treatment was continued for relatively long periods, some animals suffered recurrence during continuing undiminished treatment, as in figure 3. In earlier experiments when such tumors were harvested, passed to other animals, and retested, they were resistant to cyclophosphamide (Pla-1/CPA stem cells) but were not cross-resistant to drugs of different classes.

These results imply wide fluctuations in the ratios of Pla-1/CPA to Pla-1/0 stem cells in individual animals bearing similar tumor burdens. They also indicate that the primary type of tumor stem cells underlying the failure of cyclophosphamide treatment was T/R cells, not resting T/0 cells.

Figure 4 illustrates another important observation made by Griswold et al. Animals bearing measurable Pla-1 tumors were treated with a single 25-mg/kg dose of cyclophosphamide (about ¼ the LD_{10}). This treatment resulted in unimpressive temporary tumor regressions but dilution bioassays showed about a 5-log reduction in tumor stem cells. Tumor volume or mass behavior may underestimate tumor stem cell kill, not by a little, but by a lot. This observation has been extended and confirmed by us and others in a variety of solid tumors.

Ridgway Osteogenic Sarcoma (ROS) in AKR Mice

During the 1970s we carried out many trials in the ROS system using single drug treatment and combinations of drugs delivered in different ways (27). The growth of this tumor is nearly exponential over an unusually wide range (0.1 to almost 10 g). The doubling time is about 3 days, and cytokinetic studies imply a high proliferating fraction. This solid tumor, which is slow to metastasize, is responsive to a wide variety of drugs: alkylating agents, DNA binders, antimetabolites, and mitotic inhibitors. If the tumors are unmeasurable at the initiation of treatment, the animals are curable by various classes of drugs. But if they are > 1 g in size, antimetabolites and mitotic inhibitors fail, with tumor mass behavior curves such as in plot A in figure 5, while maximum tolerated doses of cyclophosphamide or dactinomycin will achieve high CR rates and some cures.

Optimally designed and delivered combinations of non-cross-resistant drugs are superior to single drug treatment of animals bearing 2-3-g ROS tumors. For example, optimum regimens of cyclophosphamide and 6-mercaptopurine (6-MP), delivered in an alternating manner, will provide 100% CR rates and 80%-100% cures; optimum regimens of dactinomycin plus cyclophosphamide are

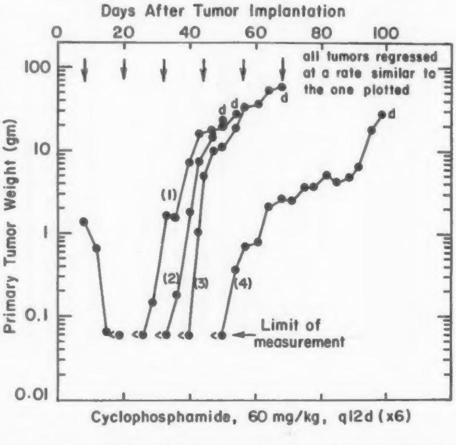


FIGURE 3.—Regression and recurrence of Pla-1 during continuing treatment with cyclophosphamide. In this trial some similarly staged and treated animals were cured. Results such as these were observed repeatedly. In earlier trials a recurring tumor was harvested, passed to other animals, and shown to be resistant to cyclophosphamide but not to other drugs of different classes.

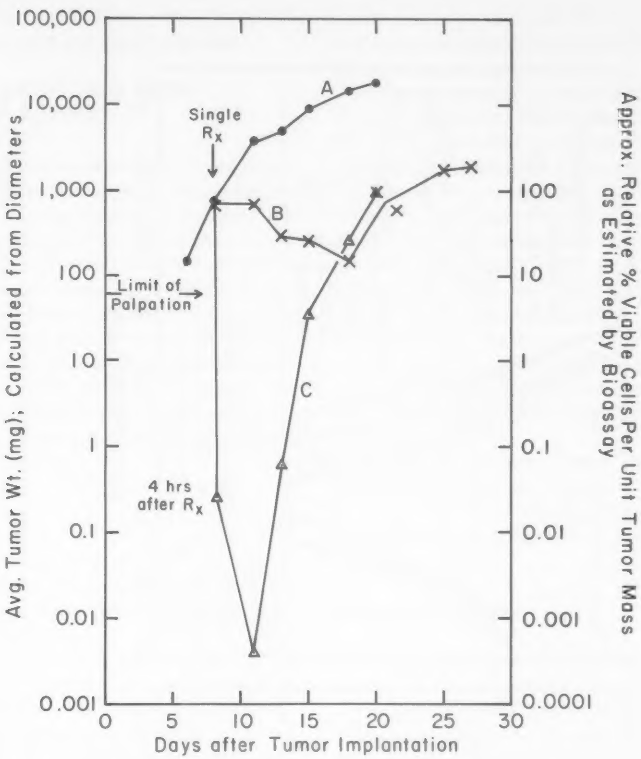


FIGURE 4.—Partial regression of Pla-1 tumors following a single 25-mg/kg dose of cyclophosphamide compared to the tumor stem cell kill and regrowth. A and B are the tumor mass behavior curves for the control and treated groups, respectively. C represents the tumor stem cell reduction and regrowth based on bioassays and related to untreated controls. Tumor mass behavior may underestimate tumor stem cell kill, not by a little but by a lot.

equally effective. On the other hand, if attention is not paid to the dose levels and dose ratios as well as the method of delivery of combinations, they may not be as effective as the maximum tolerated doses (MTD) of the individual drugs in the combination.

More specifically, many trials were carried out on the above-mentioned two-drug combinations with systematic variations in (*a*) dose levels (high doses of both drugs, high doses of one and low doses of the other, or low doses of both), and (*b*) methods of delivery (simultaneous, alternating, and sequential). Subsequent analyses of these combination trials were helpful in deducing the criteria for design of optimum two-drug combinations. These criteria include the selection of drugs, doses, and dose ratios (K_s sums and K_s ratios) and intervals between treatment that have the potential for eradicating both the T/0 and T/R stem cells without excessive host toxicity. With this number of critical variables, we find our intuition to be less than reliable and that prospective simulations are very helpful in almost any attempt to improve the design of a combination chemotherapy regimen.

Plot B in figure 5 will serve to illustrate that even a rather poor dosage regimen of cyclophosphamide plus 6-MP is more effective than an MTD of 6-MP alone. (Better results can be obtained with MTD of cyclophosphamide alone.) What was wrong with the doses used in the trial illustrated in plot B of figure 5? The cyclophosphamide dose level was too low. The low doses of cyclophosphamide would not keep the 6-MP-resistant tumor stem cells in check. In repetitive trials, animals bearing 2-3-g ROS tumors were treated in a similar alternating manner using 75-100-mg/kg doses of cyclophosphamide and 12-30-mg/kg doses of 6-MP. In these trials (six in all) 100% CR rates were achieved and the cure rate ranged between 80% and 100%.

Returning to the major theme of this paper—in our total experience with the responsive ROS, it seems clear that the primary type of tumor stem cell heterogeneity

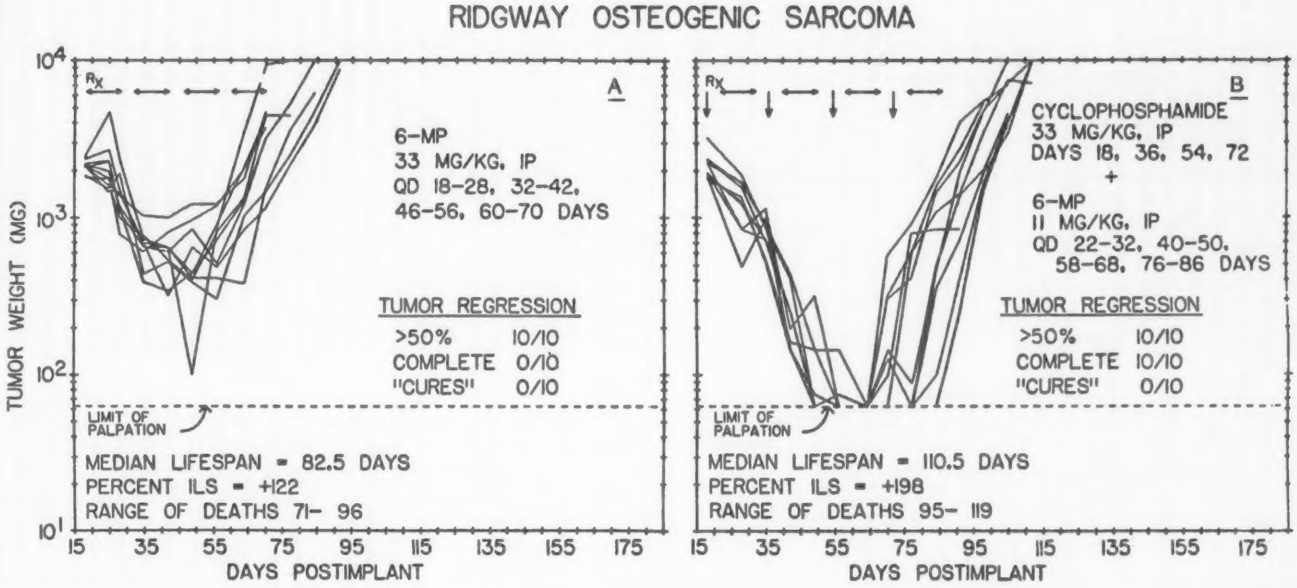


FIGURE 5.—ROS; tumor mass behavior during and after treatment with 6-MP alone and a poor selection of doses for a cyclophosphamide + 6-MP combination. Although these recurring tumors were not passed and retested for responsiveness to the drugs employed, it seems reasonable to assume that in A the failures were due to selection and overgrowth of ROS/6-MP stem cells and that in B the failures were due to the fact the doses of cyclophosphamide were too low to keep the ROS/6-MP cells in check. In other combination trials where the doses of cyclophosphamide were increased, 80%-100% cures were achieved.

responsible for chemotherapeutic failure was the initial T/R to T/0 stem cell ratio, not a low growth fraction.

RELATIVELY REFRACTORY AND VERY REFRACTORY SOLID TUMORS

One of our prejudices is that some of the biologic, kinetic, and therapeutic principles that apply to drug-sensitive neoplasms also apply (in varying degrees) to less sensitive and highly refractory neoplasms. We suspect that within the spectrum of cancers that respond so differently to chemotherapy there is a continuum of diversity (or degrees of diversity) which may be as simple, or complex, as suggested in figure 1. If this is true, then each step we can make in defining terms that we sometimes use too loosely may help in considering tumor heterogeneity and ways of circumventing limitations associated with tumor stem cell heterogeneity. Obviously, quantitative definition, when possible, is desirable.

Background

In extensive chemotherapeutic research with independently selected transplantable tumors from the same tissue of origin, Schabel, Corbett, Griswold, and associates (27-30) have observed the following.

The ranking of drugs in order of effectiveness varies markedly from tumor line to tumor line. All experimental breast tumors at similar sizes do not respond comparably to a given drug, much less to drugs of different classes. The same may be said for independently selected colon tumor lines of similar histology.

Such individual patterns of preferential responsiveness or refractoriness to a particular drug or class of drugs do *not* change notably even after many passages (eg, 25 to 50 or greater), even though the growth rate may change with repeated passage. The rather widely accepted notion that repeated transplantation of a tumor consistently leads to one that is very responsive to chemotherapy is a myth that cannot be documented.

Even after many passages the tumors in individual animals (all grown from fragments of a single tumor) may respond quite differently to identical treatment (ie, in keeping with a basic tenet of the mutation theory). This is particularly true when all tumors are relatively advanced at the initiation of chemotherapy.

Lewis Lung Carcinoma

Lewis lung carcinoma (LL) has been in passage for many years and no longer shows significant differentiation. It has a doubling time of 1-2 days in near exponential phase; the growth rate becomes markedly asymptotic as the tumor mass exceeds 1 g in size. LL is the most uniformly and rapidly metastasizing solid tumor we have worked with. By 1 week after sc implantation of 25-mg tumor fragments, LL cells have metastasized to the lungs and local resection of the primary provides no cures and little increase in survival time.

Antimetabolites or mitotic inhibitors will provide few if any CR, much less cures in animals bearing measurable LL tumors, but will cure some animals bearing small numbers of iv inoculated LL stem cells (eg, 10^3 or less).

Nitrosoureas are the most effective drugs against this neoplasm. Semustine (methyl-CCNU) will cure about one-third of the animals bearing 0.5-g tumors. Methyl-CCNU or cyclophosphamide will cure about 50% of animals bearing 10^4-10^5 iv inoculated LL stem cells. A combination of these drugs is somewhat more effective. In animals bearing 2-3-g primary LL tumors and metastatic disease, Mayo et al (31) observed that surgical resection or chemotherapy provides no cures but local resection plus methyl-CCNU and cyclophosphamide resulted in cure rates of $\geqslant$ 50%.

The results in figure 6 show the rapid selection of a methyl-CCNU-resistant subline of LL in animals bearing 0.4-g tumors. These results indicate that failure of the most effective drug against LL is due to T/R cells, not to resting T/0 cells.

Mammary Tumors

Corbett and Griswold et al (28,30) placed into transplantation ten independent mammary tumor lines arising in C3H/He female mice and determined their responsiveness to different classes of anticancer agents. None were curable by chemotherapy alone after they had reached measurable size and the degrees of responsiveness to different classes of drugs differed between tumor lines.

Mammary adenocarcinoma 16/C responded best to doxorubicin and related drugs. Figure 7 shows that doxorubicin provided PR and CR followed by tumor progression during continuing treatment. Figure 7 indicates that a simultaneously delivered combination of CAF (cyclophosphamide, doxorubicin, and 5-FU) was somewhat better, but the animals suffered relapse during combination treatment. When fragments of one of the tumors that recurred during CAF treatment were passed to other animals and tested for resistance to each of the drugs in the combination, the tumor cells proved to be clinically resistant to doxorubicin but not to cyclophosphamide or 5-FU. These results imply that the most effective drug in the combination delayed or prevented overgrowth of phenotypes resistant to the less effective drugs but the less effective drugs did not prevent overgrowth of doxorubicin-resistant mammary tumor cells. They also point to the design changes that would be required to improve combination treatment.

In this relatively refractory tumor it was the T/R stem cells, not the T/0 stem cells, that were limiting with respect to cure by the most effective drug available for

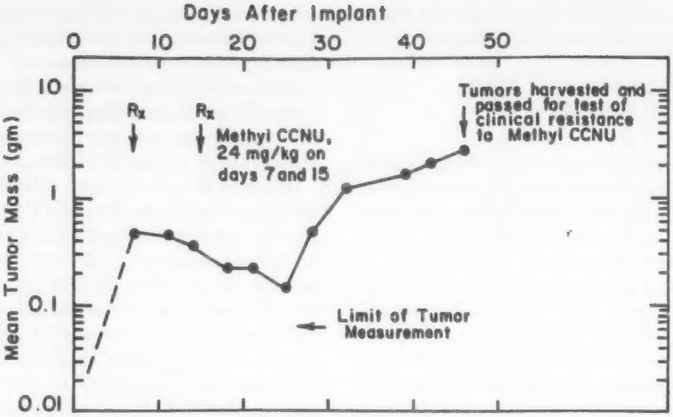


FIGURE 6.—Selection of a methyl-CCNU-resistant subline of LL. When tumors were harvested and fragments were passed to other animals, they proved to be markedly resistant to methyl-CCNU but were not cross-resistant to cyclophosphamide.

treating the mammary tumor.

Colon Tumors

Corbett et al (29) also undertook the arduous task of placing four carcinogen-induced colon tumors into passage and testing their responsiveness to a wide variety of drugs. Again they observed marked differences in the degrees of responsiveness of the different tumor lines to different drugs. For example, Colon tumor 36 was quite responsive to palmO-ara-C, a slow release form of ara-C. Colon tumor 38 responded rather poorly to this S-phase-specific drug, and Colon tumor 26 showed essentially no response to MTD (*see* fig 8).

Simpson-Herren et al (32) carried out studies on the growth rates and cytokinetic behavior of Colon tumors 36, 38, and 26. The PLM curves and the labeling index curves with increase in tumor size and age seemed consistent with the tumor response, Colon 36 > 38 > 26, but so did the relative capacities of the three tumors to phosphorylate deoxycytidine.[3]

Figures 9 and 10 will serve to illustrate the excellent response of Colon tumor 36 to palmO-ara-C when the tumors are small, but highly fluctuating degrees and durations of response in individuals when the tumors are much larger. In Figure 10 note varying degrees of response of tumors in individuals and tumor progression in most during continuing undiminished treatment. These differences imply fluctuating ratios of T/ara-C to T/0 stem cells in similar-sized tumors at the initiation of treatment. It is conceivable that the relative response of the three colon tumors to palmO-ara-C (Colon tumor 36 > 38 > 26) is due to an initial reverse order of T/ara-C to T/0 stem cell ratios in tumors of the same size.

Figure 11 shows the response of one of Corbett's colon tumors (Colon 07/A) to dacarbazine (DTIC); the others showed little significant response to this agent. As can be seen, repetitive doses of DTIC caused regression of small Colon 07/A tumors, but all resumed growth during continuing treatment. One of these recurring tumors was passed to other animals and tested for responsiveness to DTIC; it was observed to be resistant. These results point to the type of tumor stem cell heterogeneity underlying failure of DTIC in treatment of an experimental colon tumor. It is possible that the colon tumors which responded poorly to DTIC contained higher T/DTIC to T/0 tumor stem cell ratios than Colon tumor 07/A.

B16 Melanoma

During the 1970s Griswold (30) carried out many quantitative chemotherapeutic trials and ancillary experiments using the B16 melanoma system. Briefly, he showed that B16 melanoma is very refractory to all classes of available anticancer drugs and to many two-drug combinations. If the tumor is > 0.5 g in size, PR are rare and CR are not achieved with MTD of any drug. Chemotherapeutic cures were consistently achieved only when the tumor stem cell burden was about $\leqslant 10^3$. As would be expected, this is not a sharp cut-off point; on occasion, some animals in groups bearing an estimated 10^4 tumor stem cell burden were cured by an MTD of methyl-CCNU, the best available drug for treating animals bearing this tumor.

---

[3] It is well-established that L1210/ara-C cells are resistant to ara-C because of their loss of deoxycytidine kinase activity—the same enzyme that converts ara-C to its cytotoxic form.

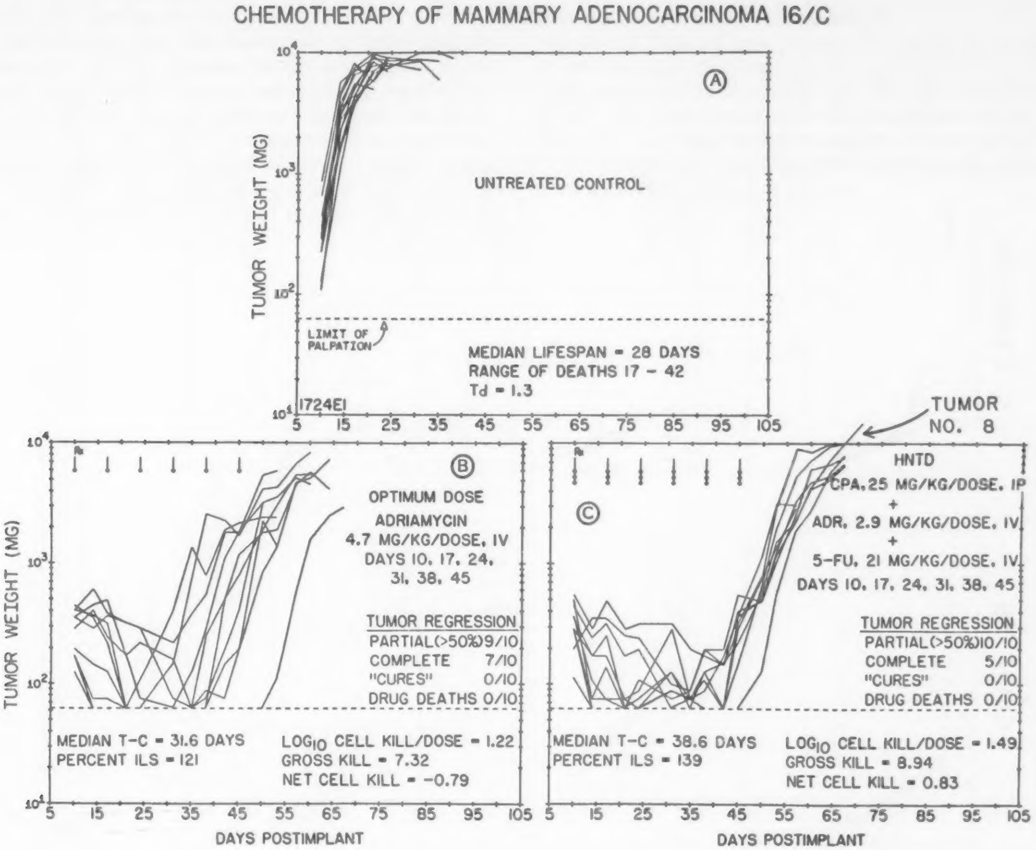


FIGURE 7.—Mammary adeocarcinoma 16/C; response to doxorubicin and to a 3-drug combination, CAF. *Plot A*—untreated controls; *Plot B*—MTD of doxorubicin alone: 4.7 mg/kg/dose, q7d (x 6), starting on Day 10; *Plot C*—maximum tolerated courses of cyclophosphamide, doxorubicin, and 5-FU given simultaneously q7d (x 6) starting on Day 10. When tumor No. 8 was harvested and passed to other animals, it proved to be resistant to doxorubicin but not to cyclophosphamide or 5-FU.

If we turned back to Holland's classification of human cancers by chemotherapeutic effect (table 1) and extrapolated to this experimental tumor, we would have to place B16 melanoma in the precurable category.[4]

The doubling time of B16 melanoma in near exponential phase is 1-2 days, but the growth rate becomes notably asymptotic when the tumors exceed 1 g in size. After long transplantation, this refractory tumor retains one characteristic of the tissue of origin. It still produces melanin, although we cannot see why this biochemical characteristic per se would contribute to the tumor being so refractory to chemotherapy.

Griswold et al carried out experiments to answer this question. Do small B16 melanoma tumors contain stem cells that are specifically and permanently resistant to methyl-CCNU, the most effective drug against this neoplasm? Animals bearing unmeasurable tumors (< 0.1 g) were treated with a single LD_{10} of methyl-CCNU and after tumors appeared (with some delay compared to controls) they were harvested, passed to other animals, and tested for resistance to methyl-CCNU. They were significantly resistant. After two passages in treated animals the tumors showed almost no response to an LD_{10} of methyl-CCNU. Later these B16/methyl-CCNU tumors were repeatedly passed in untreated animals; they retained their resistance to methyl-CCNU but were not significantly cross-resistant to cyclophosphamide. These results imply that the T/methyl-CCNU to T/0 stem cell ratio is relatively high in B16 melanoma as compared to the

---

[4] In fact, a number of the transplantable tumors (breast and colon) already mentioned also would have to be classed as precurable by chemotherapy except after resection of the primary tumor.

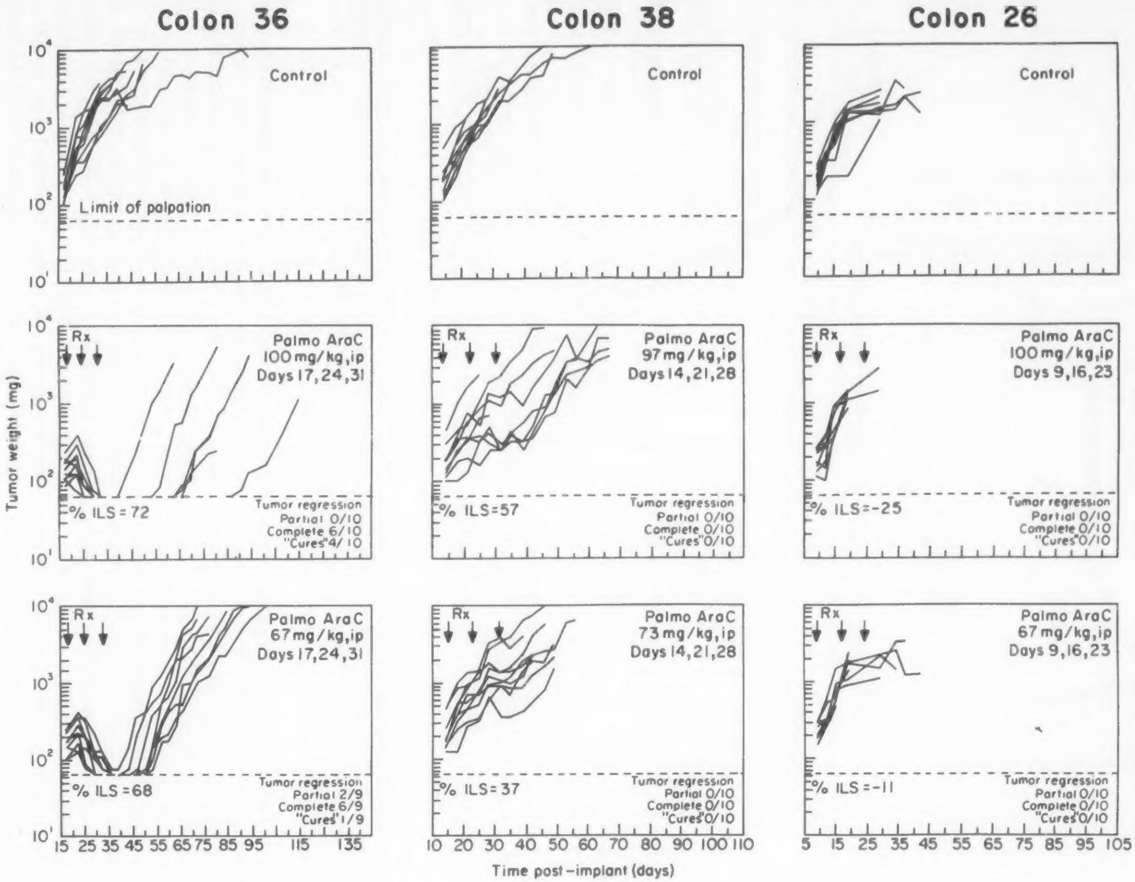


FIGURE 8.—Comparison of the response of Colon tumors 36, 38, and 26 to palmO-ara-C (*from* ref 32 with permission of author and publisher). These and many additional data show that the responsiveness of these 3 tumors to palmO-ara-C (a slow release form of ara-C) is in this order: Colon 36 > 38 > 26. It seems likely that the T/ara-C to T/0 stem cell ratios in these colon tumors are in the reverse order: Colon 26 > 38 > 36. The tumor mass curves are terminated at death of the host.

more responsive murine leukemias.

Experiments were then carried out in which animals bearing 0.4-g B16 melanoma tumors were treated with a wide range of single doses of methyl-CCNU (eg, 0.5 LD_{10} to supralethal doses of three to four times the LD_{10}). Twenty-four hours later the tumors were harvested and passed to untreated animals for dilution bioassay of surviving tumor stem cells. The supralethal doses (three to four times the LD_{10}) "sterilized" the tumor stem cells in the 0.4-g tumors; the lower doses did not.

Although these are not "practical" experiments, they do serve to show that toxic doses of methyl-CCNU provided exposures that will kill the T/0 and T/methyl-CCNU stem cells in measurable B16 melanoma tumors. Many other experiments had shown that MTD of methyl-CCNU provide only temporary minimum responses and a few PR (no CR) in tumors of this size.

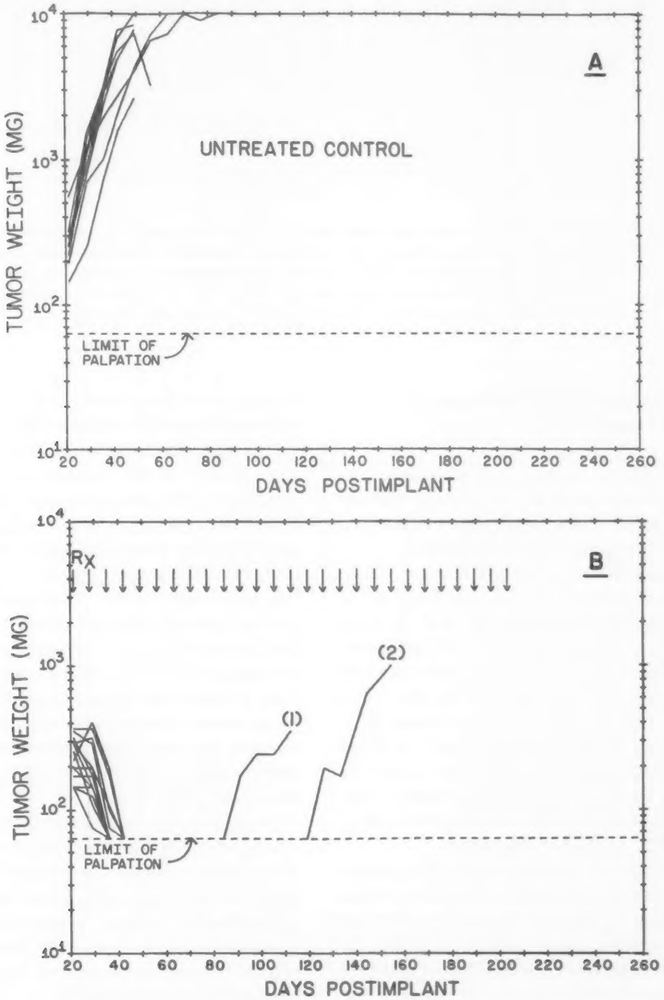


FIGURE 9.—Long-term treatment of animals bearing small Colon 36 tumors with palmO-ara-C. A = untreated controls; B = treated with palmO-ara-C, 63 mg/kg/dose, q7d (x 27) starting on Day 21 after subcutaneous implantation of small trocar fragments. Drug deaths, 2 of 15; CR, 13 of 15; cures, 11 of 15; and recurrences, 2 of 15. Tumors from the 2 animals that suffered recurrence during continuing undiminished treatment were harvested before death, passed to other animals, and observed to be resistant to ara-C.

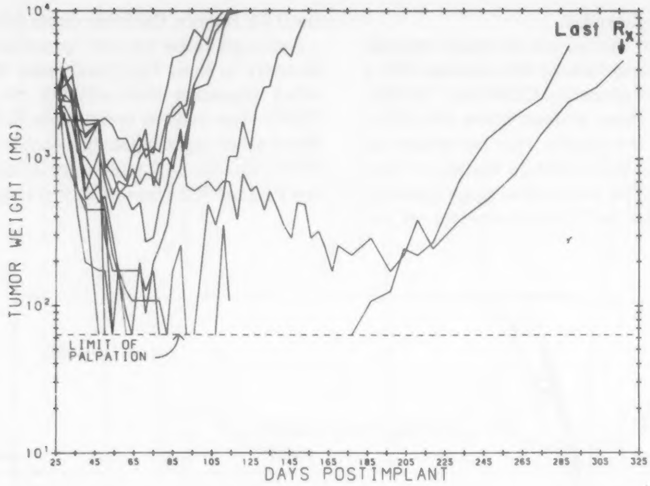


FIGURE 10.—The diversity in both degree and duration of response of 2-3-g Colon 36 tumors on long-term treatment of animals with palmO-ara-C. PalmO-ara-C was administered in qd (x 9) courses at 19 mg/kg/dose, with 10-day intervals of rest between, until death or for 313 days. Drug deaths, 2 of 14; minimum responses, 1 of 14; PR, 5 of 14; CR, 3 of 14; cures, 3 of 14. There was tumor progression after regressions in all animals, except those cured. The increase in survival time over untreated controls was 179%.

Classification of Experimental Neoplasms by Chemotherapeutic Effect

From raw data considered in the informal report mentioned in the introduction, we classified a number of experimental neoplasms by chemotherapeutic effect (*see* table 4). All but one were transplanted neoplasms.

The basis of this classification is indicated below.

Responsive to chemotherapy.—Neoplastic diseases for which the best available chemotherapy will provide ≥ 50% cures of animals bearing about 10^6-10^8 malignant stem cells or solid tumors of ≥ 1 g. In those animals cured by single drug treatment it may be deduced that such burdens contained no tumor stem cells that were resistant to tolerated doses of the drug employed. In those animals cured by combination chemotherapy it may be concluded that such burdens contained no tumor stem cells that were resistant to all of the drugs in the combination as delivered (T/R_1, $T/R_{1,2}$, or T/R_n cells).

Moderately refractory to chemotherapy.—Neoplastic diseases for which the best available chemotherapy fails to cure animals bearing 1-g tumors (stem cell burden not clearly established). In these tumors usually only MTD of one class of drug will regularly cure significant numbers of animals bearing measurable tumors, and failure of the best drugs is consistently found to be due to overgrowth of T/R cells.

Very refractory to chemotherapy.—Neoplastic diseases for which no available drug will consistently cure animals bearing 25-50-mg tumors, and the best available drug will provide no CR and few PR in animals bearing measurable tumors. These neoplasms are curable by chemotherapy only if the animals are bearing no more than about 10^3-10^4 tumor stem cells. B16 melanoma is an example of a transplantable neoplasm in the very refractory category; certain transplantable colon and breast tumors also fit into this category.

In some respects the classification of experimental cancers by chemotherapeutic effect (table 4) is similar to Holland's classification of human cancers by chemotherapeutic effect; it differs in other respects.

(*a*) In both there is an assumed inverse relationship between tumor stem cell burden and curability with chemotherapy, but with large differences in the maximum burden curable in different cancers. For both this seems a rather safe assumption.

(*b*) In animals (mice) the lethal burden of leukemia cells is about 10^9 and solid tumors almost never exceed 10 g, about one-half the weight of the mouse. Only tumors that are slow to metastasize will reach 10 g before killing the host. Mouse "tetracytomas" (10^{12}) are a biologic impossibility. It is probable that a rather high percentage of the cells in animal and human tumors of > 1 g do not retain stem cell capacity.

(*c*) Since all of the experimental neoplasms in table 4 are transplantable except one, and most no longer show discernible evidence of differentiation, we cannot provide a breakdown of responsiveness to chemotherapy by de-

TABLE 4. -- Classification of experimental neoplasms by chemotherapeutic effect

(All transplanted neoplasms except one)

	A. Responsive	B. Moderately refractory	C. Very refractory
Curability when the tumor stem cell burden is 10^6-10^8 or the tumor mass is 1.0 gram or >:	≥ 50%	< 50%	Uncommon
Morphology:	Undifferentiated	Undifferentiated	Undifferentiated to poorly differentiated
Doubling time (< 1 gm):[*]	0.4-3.4 days	1-5 days	1->6 days
Growth fraction:[†]	Relatively high	Relatively high	Generally lower (?)
Influence of size:	Critical	Critical	Critical
T/R to T/0 ratio:[‡]	Low	Higher	Higher
	1. L1210 leukemia	1. Spontaneous AK leukemia[§]	1. B16 melanoma
	2. P388 leukemia	2. Lewis lung carcinoma	2-11. Ten independently selected mammary tumors
	3. Long passage AK leukemia	3. Colon 36	
	4. First passage AK leukemia		12-14. Three independently selected colon tumors
	5. Plasmacytoma (Pla-1)		
	6. Ridgway osteogenic sarcoma		
Type of tumor stem cell heterogeneity primarily responsible for the failure of the best available chemotherapy for the particular neoplasm	Singly or doubly drug-resistant mutant stem cells (T/R cells)	Singly or doubly drug-resistant mutant stem cells (T/R cells)	Singly or doubly drug-resistant mutant stem cells where studied (B16/methyl CCNU; mammary 16C/adriamycin; Colon 26/methyl CCNU; Colon 07A/DTIC)

[*] The doubling times of all of the solid tumors, excepting ROS, increased markedly after they exceeded 1 gram in size.

[†] Cytokinetic data not available on some of the solid tumors.

[‡] Additional confirmation needed.

[§] Two factors are responsible for spontaneous AK leukemia being in this classification: the extreme advancement at diagnosis and the fact that the host is carrying the Gross virus which will reinduce leukemia if the leukemia stem cells are eradicated.

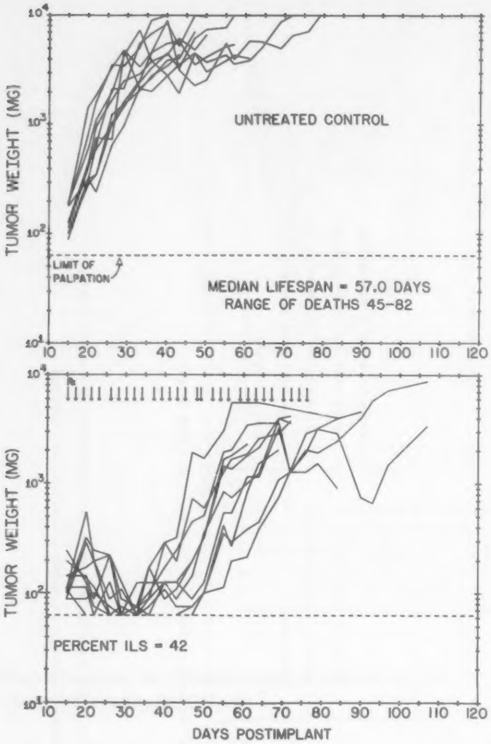


FIGURE 11.—Colon adenocarcinoma 07/A; tumor regression and recurrence during continuing treatment with DTIC. A tumor that regressed and regrew during DTIC treatment was harvested, passed, and tested for resistance to DTIC. As expected, it proved to be resistant.

gree of differentiation. Nevertheless, we note that some of the transplantable breast and colon tumors and B16 melanoma respond no better than Holland's subcurable and precurable human cancers when essentially the same criteria are used. Furthermore, the very refractory tumors respond poorly even when they are < 0.1 g in size.

(*d*) Some of the cytokinetic parameters of the experimental and human cancers being considered overlap (eg, labeling index and average cell cycle time) but the median doubling times of the transplantable animal cancers in near exponential phase are generally much shorter (eg, 0.4 day to 4 days versus somewhat < 4 days to, say, ≥ 50 days).

(*e*) In the experimental cancers we can be certain of the type of tumor stem cell heterogeneity responsible for failure of the best available chemotherapy. As has been emphasized throughout this paper, whenever responsive, moderately refractory, and very refractory malignancies respond temporarily and then resume growth during continued treatment with the same doses and schedule of a drug, the surviving tumor stem cells are invariably resistant to the drug used. When this occurs during combination chemotherapy, the recurring tumors show resistance to one or more but not necessarily to all of the individual drugs in the combination. The same phenomena presumably underlie chemotherapeutic failure when drugs are employed in an adjuvant setting as well.

CONCLUSION

In closing, let us turn back to *Questions A* and *B*. *Question A* no longer seems so puzzling in view of extensive analyses showing the influence of effective but noncurative chemotherapy on survival time variance and observations of widely fluctuating degrees of response of individual solid tumors of a given type during long-term treatment. This is what should be expected if we accept a basic tenet of the somatic mutation theory.

Question B regarding the phenomena underlying "classification of cancers by chemotherapeutic effect" is a broader and more difficult one. But after reviewing many data gained on treating animals bearing neoplasms that are responsive, relatively refractory, and very refractory to chemotherapy (ie, to some drugs or all drugs), we are beginning to wonder if this is not an answerable question. It appears that marked differences in the T/R to T/0 tumor stem cell ratios between different types of cancer (of a given size) may underlie both experimental and clinical classifications of cancer by chemotherapeutic effect. If this is true, what phenomena might account for higher T/R to T/0 ratios in refractory cancers compared to chemotherapeutically responsive cancers? The two most obvious are higher mutation rates to a resistant state in the refractory cancers, or some sort of "natural selection" of diverse T/R stem cells in the refractory cancers.

REFERENCES

1. HOLLAND JF. Chemotherapy of gigacytomas: the treatment and curability of "solid" tumors with drugs. *In* Cancer Achievements, Challenges and Prospects for the 1980's (Burchenal JH, and Oettgen HF, eds). New York, Grune & Stratton, 1981, vol 2, pp 563-583.
2. ______. Breaking the cure barrier. Karnofsky memorial lecture. J Clin Oncol 1(2):75-90, 1983.
3. DEVITA VT, JR. The James Ewing Lecture: the relationship between tumor mass and resistance to chemotherapy: implications for surgical adjuvant treatment of cancer. Cancer 51:1209-1220, 1983.
4. SKIPPER HE. The forty-year-old mutation theory of Luria and Delbruck and its pertinence to cancer chemotherapy. *In* Advances in Cancer Research. San Diego, Academic Press, 1983, vol 40, pp 331-363.
5. GOLDIE JH, and COLDMAN AJ. A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 63:1727-1733, 1979.
6. LURIA SE, and DELBRUCK M. Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28:491-511, 1943.
7. GOLDIE JH, and COLDMAN AJ. Quantitative model for multiple levels of drug resistance in clinical tumors. Cancer Treat Rep 67:923-931, 1983.
8. MACKILLOP WJ, CAIMPI A, TILL JE, ET AL. A stem cell model of human tumor growth: implications for tumor cell clonogenic assays.

JNCI 70:9-16, 1983.

9. GOLDIE JH, and COLDMAN AJ. Clinical implications of the phenomenon of drug resistance. *In* Drug and Hormone Resistance in Neoplasia. Clinical Concepts (Bruchovsky N, and Goldie JH, eds). Boca Raton, FL, CRC Press, 1983, vol II, pp 111-127.
10. BURCHENAL JH, CREMER MA, WILLIAMS BS, ET AL. Sterilization of leukemia cells in vivo and in vitro. Cancer Res 11:700-705, 1951.
11. SCHABEL FM, JR, SKIPPER HE, TRADER MW, ET AL. Experimental evaluation of potential anticancer agents. XIX. Sensitivity of nondividing leukemic cell populations to certain classes of drugs in vitro. Cancer Chemother Rep 48:17-30, 1965.
12. BURCHENAL JH, ROBINSON E, JOHNSTON SF, ET AL. The induction of resistance to 4-amino-N^{10}-methyl-pteroylglutamic acid in a strain of transmitted mouse leukemia. Science 111:116-117, 1950.
13. LAW LW. Resistance in leukemic cells to an adenine antagonist, 6-mercaptopurine. Proc Soc Exp Biol Med 84:409-412, 1953.
14. HUTCHISON DJ, and SCHMID FA. Cross-resistance and collateral sensitivity. *In* Drug Resistance and Selectivity, Biochemical and Cellular Basis. New York, Academic Press, Inc, 1973, pp 73-126.
15. SKIPPER HE. Cancer Chemotherapy, Vol 1: Reasons for Success and Failure in Treatment of Murine Leukemias with the Drugs Now Employed in Treating Human Leukemias. Ann Arbor, MI, University Microfilms, Int, 1976, 166 pp.
16. BROCKMAN RW. Resistance to therapeutic agents. *In* Cancer Achievements, Challenges and Prospects for the 1980's (Burchenal JH, and Oettgen HF, eds). New York, Grune and Stratton, 1981, vol 2, pp 55-66.
17. SCHMID FA, HUTCHISON DJ, OTTER GM, ET AL. Development of resistance to combinations of six antimetabolites in mice with L1210 leukemia. Cancer Treat Rep 60:23-27, 1976.
18. SCHABEL FM, JR, SKIPPER HE, TRADER MW, ET AL. Drug control of ara-C-resistant tumor cells. Med Pediatr Oncol 1:125-148, 1982.
19. SKIPPER HE, SCHABEL FM, JR, and WILCOX WS. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemias. Cancer Chemother Rep 35:1-111, 1964.
20. BRUCE WR, MEEKER BE, and VALERIOTE FA. Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. JNCI 37:233-245, 1966.
21. VAN PUTTEN LM. Are cell kinetic data relevant for the design of tumor chemotherapy schedules? Cell Tissue Kinet 7:493-504, 1974.
22. BRUCE WR, MEEKER BE, POWERS WE, ET AL. Comparison of the dose and time-survival curves for normal hematopoietic and lymphoma colony-forming cells exposed to vinblastine, vincristine, arabinosylcytosine, and amethopterin. JNCI 42:1015-1023, 1969.
23. LAW LW. Origin of the resistance of leukaemic cells to folic acid antagonists. Nature 169:628-629, 1952.
24. SCHABEL FM, JR, SKIPPER HE, TRADER MW, ET AL. Spontaneous AK leukemia (lymphoma) as a model system. Cancer Chemother Rep 53:329-344, 1969.
25. SKIPPER HE, SCHABEL FM, JR, TRADER MW, ET AL. Response to therapy of spontaneous, first passage, and long passage lines of AK leukemia. Cancer Chemother Rep 53:345-366, 1969.
26. GRISWOLD DP, JR, SCHABEL FM, JR, WILCOX WS, ET AL. Success and failure in the treatment of solid tumors. I. Effects of cyclophosphamide (NSC-26271) on primary and metastatic plasmacytoma in the hamster. Cancer Chemother Rep 52:345-387, 1968.
27. SCHABEL FM, JR, GRISWOLD DP, JR, CORBETT TH, ET AL. Testing therapeutic hypotheses in mice and man: observations on the therapeutic activity against advanced solid tumors of mice treated with anticancer drugs that have demonstrated or potential clinical utility for treatment of advanced solid tumors of man. *In* Cancer Drug Development, Part B. Methods in Cancer Research (H Busch, and DeVita VT, Jr, eds). New York, Academic Press, Inc, 1979, vol 17, pp 3-51.
28. CORBETT TH, GRISWOLD DP, JR, ROBERTS BJ, ET AL. Biology and therapeutic response of a mouse mammary adenocarcinoma (16/C) and its potential as a model for surgical adjuvant chemotherapy. Cancer Treat Rep 62:1471-1488, 1978.
29. CORBETT TH, GRISWOLD DP, JR, ROBERTS BJ, ET AL. Evaluation of single agents and combinations of chemotherapeutic agents in mouse colon carcinomas. Cancer 40:2660-2680, 1977.
30. GRISWOLD DP, JR. The potential for murine tumor models in surgical adjuvant chemotherapy. Cancer Chemother Rep 5:187-204, 1975.
31. MAYO JG, LASTER WR, JR, ANDREWS CM, ET AL. Success and failure in the treatment of solid tumors. III. "Cure" of metastatic Lewis lung carcinoma with methyl-CCNU (NSC-95441) and surgery-chemotherapy. Cancer Chemother Rep 56:183-195, 1972.
32. SIMPSON-HERREN L, CORBETT TH, and GRISWOLD DP. The cell population kinetics and response to an S-phase specific agent of three transplantable colon tumor lines. Cell Tissue Kinet 13:613-624, 1980.

The Preclinical New Drug Research Program of the National Cancer Institute

John S. Driscoll[1,2,*]

The discovery and development of anticancer drugs with clinical potential are the responsibility of the Developmental Therapeutics Program (DTP), Division of Cancer Treatment, National Cancer Institute (NCI). Approximately 10,000 compounds/year are selectively acquired and screened against murine tumor models in order to discover new, active materials. The program required to accomplish this objective, as well as the subsequent tasks of formulation development and toxicology testing, is described. Since its inception in 1955, the preclinical new drug research program of the NCI has played a major role in the discovery and development of new agents which have been entered into clinical trial. The NCI has been responsible for the discovery of eight of the 16 commercially available drugs discovered since 1955. In addition, the NCI has played an important role in the clinical evaluation of all 16 of these New Drug Application (NDA)-approved drugs. During 1977–1982, the NCI filed Investigational New Drug Applications (INDA) for 33 cytotoxic agents. It was responsible for the discovery of the antitumor activity of 73% of these compounds. Most of the INDA compounds were acquired directly through NCI efforts. The DTP active acquisition program was responsible for obtaining 69% of these materials, with an additional 12% coming from the DTP intramural research program. Only 19% were received as voluntary submissions. The DTP active acquisition and screening effort is shown to have played even a larger role in identifying and obtaining those compounds which are currently in earlier stages of the NCI drug discovery and development process. [Cancer Treat Rep 68:63–76, 1984]

The discovery and preclinical development of anticancer drugs with potential clinical utility are the responsibility of the Developmental Therapeutics Program (DTP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI). While only the preclinical component of the NCI's new drug program will be described in this review, the clinical component is of equal importance, since it ultimately provides the only important information about any new drug, ie, its utility in humans. The DCT is the organizational component of the NCI responsible for both the clinical and preclinical new drug programs.

Drug development is a general term which often is used to cover all phases of a new drug project—from discovery through clinical trial. A description of this process is somewhat more accurate, however, if the general, all-encompassing term is "new drug research," and the term "drug development" is reserved for one component of the new drug research process. New drug research may be divided conveniently into two preclinical phases (discovery and development) and a clinical trial phase (fig 1). The integration of functions within the phases is shown in more detail in figure 2. Often thought to be synonymous with new drug research is the "screening program." However, the screening program is but one part of the new drug program (fig 2). It is an especially important part because biologic screening is the NCI's major effort to discover *new leads* to clinically useful agents. This review is concerned with the preclinical aspects of the way the DTP achieves the various new drug stages (objectives) using the functions (procedures) indicated in figure 1.

---

[1] Developmental Therapeutics Program, Division of Cancer Treatment (DCT), National Cancer Institute, Bethesda, MD.

[2] I thank Dr. Saul A. Schepartz, Deputy Director, DCT, for the large amount of well-organized reference data he made available to me, and Ms. Emily Olausen for her assistance with the preparation of the manuscript.

[*] *Reprint requests to:* John S. Driscoll, PhD, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bldg 37, Rm 6D24, Bethesda, MD 20205.

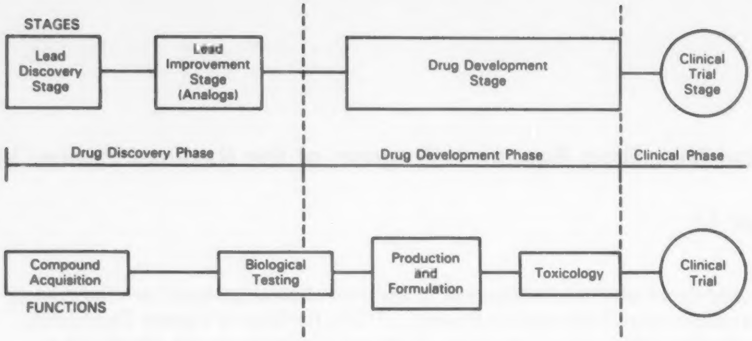

STAGES
Lead Discovery Stage
Lead Improvement Stage (Analogs)
Drug Development Stage
Clinical Trial Stage
Drug Discovery Phase
Drug Development Phase
Clinical Phase
Compound Acquisition
Biological Testing
Production and Formulation
Toxicology
Clinical Trial
FUNCTIONS

FIGURE 1.—Drug research phases: stages vs functions involved.

THE NCI NEW DRUG PROGRAM

Background

The NCI new drug program began as the Cancer Chemotherapy National Service Center (CCNSC) in 1955 and, since that time, this program has evaluated > 370,000 synthetic compounds and pure natural products for antitumor activity against a variety of animal tumor models. A number of these compounds have gone on to clinical trial.

The early history of cancer drug research and the organization of the NCI program have been described in a comprehensive 1979 review article (1). While a few details have changed during the intervening time period, the general information is still applicable and only selected items, especially pertinent to the preclinical aspects of the program, will be expanded upon here.

The DTP is organized into seven extramural Branches which are responsible for initiating and administering the contracts which perform the functions shown in figure 1 and for establishing relationships with industry. These Branches are: Drug Synthesis and Chemistry, Natural Products, Drug Evaluation, Animal Genetics and Production, Pharmaceutical Resources, Toxicology, and Information Technology. They utilize > 100 contractors to accomplish the preclinical objectives of the 35 million dollar/year DTP new drug program. In addition, an eighth Branch, Extramural Research and Resources, administers an NCI grant program in pharmacology and biochemistry with approximately equal funding. Also important to DTP cancer research, but not formally connected with the new drug program, are five DTP intramural laboratories which conduct research in facilities located at the National Institutes of Health (NIH) in Bethesda, MD.

Operational Organization

The DTP and DCT are organized into operational groups which cross administrative lines and ensure (*a*) rapid analysis of all new data, (*b*) prompt decisions regarding selection of the most promising new compounds, and (*c*) follow-up on the progress of those functions which must be completed prior to Investigational New Drug Application (INDA) filings with the Food and Drug Administration (FDA). This operational process is shown in figure 3. The functions of these operational units have been described previously (1). Each unit in the chain plays a key role in the progress of a new drug, since all are linked in series, with the exception of the Operating Committee which is in parallel

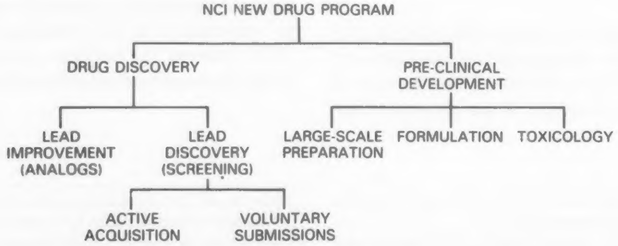

NCI NEW DRUG PROGRAM
DRUG DISCOVERY
PRE-CLINICAL DEVELOPMENT
LEAD IMPROVEMENT (ANALOGS)
LEAD DISCOVERY (SCREENING)
LARGE-SCALE PREPARATION
FORMULATION
TOXICOLOGY
ACTIVE ACQUISITION
VOLUNTARY SUBMISSIONS

FIGURE 2.—Functional organization of the NCI new drug program.

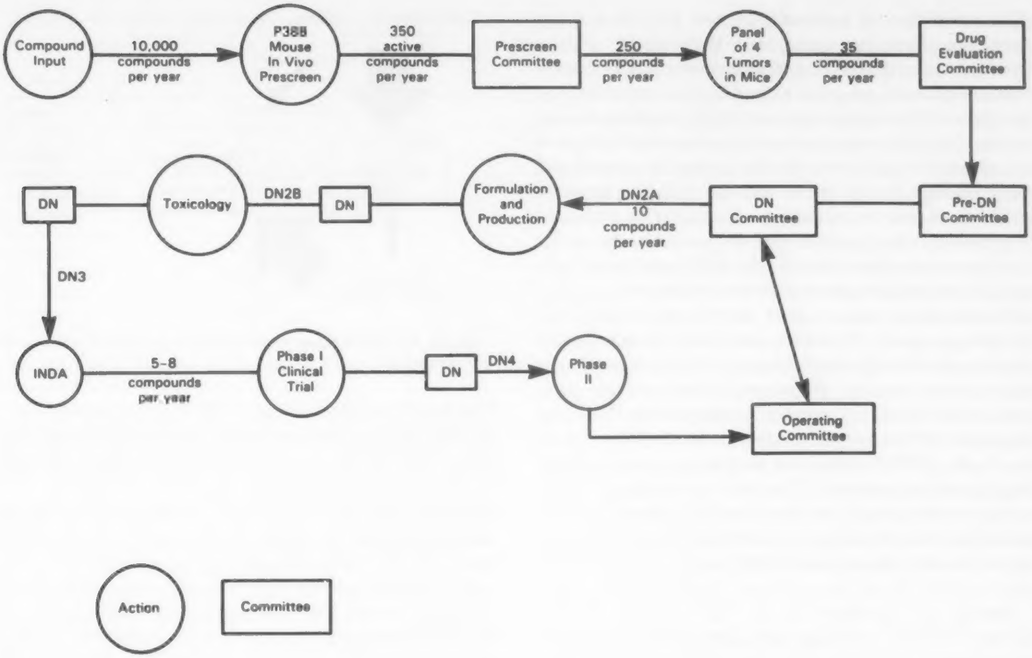

Compound Input
10,000 compounds per year
P388 Mouse In Vivo Prescreen
350 active compounds per year
Prescreen Committee
250 compounds per year
Panel of 4 Tumors in Mice
35 compounds per year
Drug Evaluation Committee
Pre-DN Committee
DN Committee
DN2A
10 compounds per year
Formulation and Production
DN
DN2B
Toxicology
DN
DN3
INDA
5-8 compounds per year
Phase I Clinical Trial
DN
DN4
Phase II
Operating Committee
Action
Committee

FIGURE 3.—DCT drug discovery and development process.

across the steps in the Decision Network (DN) process.

The DN Committee, composed of 28 senior DCT scientists and oncologists, is responsible for evaluating the data developed for new clinical trial candidates and for making recommendations to the Director, DCT, as to whether a compound should be advanced to the next stage in the DN process. Figure 4 shows a simplified schematic for the three preclinical DN steps.

It is the responsibility of the Operating Committee, which is composed of 12 DCT extramural senior staff, to track and implement the rapid progress of each of the approximately 50 compounds in DN categories 2A, 2B, and 3, ie, in the drug development phase of the new drug program (figs 1 and 2).

Drug Discovery Phase

The drug discovery phase of a new drug project (fig 1) is composed of two stages (fig 2): new lead discovery and lead improvement (analogs). A lead compound is the first member of a series discovered to be active. An analog results from a structural modification of the lead compound prepared in an attempt to improve the pharmacologic or physical properties of the lead.

Lead Improvement (Analogs)

A few examples can be used to illustrate the lead improvement process.

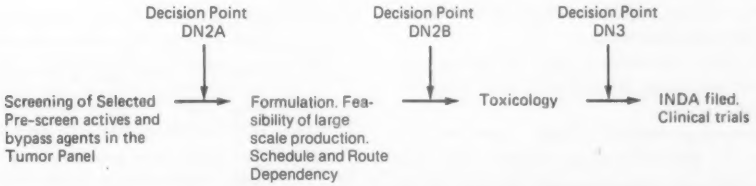

Decision Point DN2A
Decision Point DN2B
Decision Point DN3
Screening of Selected Pre-screen actives and bypass agents in the Tumor Panel
Formulation. Feasibility of large scale production. Schedule and Route Dependency
Toxicology
INDA filed. Clinical trials

FIGURE 4.—Simplified DCT DN schematic.

Daunorubicin was isolated as a new lead from a fermentation screening program. Doxorubicin (Adriamycin), a structural analog of daunorubicin, was discovered in a planned program of lead improvement using a mutation of the micro-organism which produces daunorubicin. Cisplatin was a serendipitously discovered new lead. Planned lead improvement studies have produced many analogs in this series and the NCI has screened > 1200 platinum compounds in a search for an improved drug. Several new platinum analogs have been chosen for development to clinical trial by the NCI, based on activity against cisplatin-resistant tumors or reduced toxicity. Anthracenedione was a lead discovered in the NCI screening program. This lead compound was quickly improved upon through synthetic modification to produce a more potent analog, dihydroxyanthracenedione (mitoxantrone), which is presently in clinical trial. While the major goal of the screening program is the discovery of new leads, DTP also explores analogs in a balanced approach to drug discovery. This includes an intense effort to discover what might be called "early" analogs. An analog of this type would have neither multiple members in early clinical trial nor a well-established clinical parent drug.

Analogs are developed by the NCI program if they attempt to solve a problem associated with an existing clinical agent. Increased activity and/or decreased limiting toxicity are obvious reasons for analog investigation. DTP emphasis in the drug discovery phase, however, is on new lead discovery. Lead discovery is very high-risk research compared with analog development. While private organizations often adequately cover analog development for compounds on which they have a patent position, the amount of industrial, lead discovery work is relatively small for antitumor agents.

Lead Discovery

There are two approaches to new lead discovery, screening and rational drug design. The logical question arises concerning the utilization of screening if drugs can be intellectually designed. Screening and drug design are different tools used to do different jobs (fig 5). Although new lead compounds are occasionally designed based on theoretic considerations, screening is the major tool in lead *discovery* while drug design is an important tool in lead *improvement*. It should be noted that the discovery of new natural product leads always is accomplished through screening.

There are two major variables in any screening program, the biologic screening models used and the compounds chosen for testing.

a. Biologic screening models.—There has been a steady evolution of NCI screening models since the founding of the NCI new drug program in 1955 (table 1). The screening program and the tumor models used have been described in detail (2). The performance of a number of standard anticancer agents against the 1975 tumor panel models is shown in table 2. Many of these standard agents had a broad spectrum of activity. Only bleomycin was inactive in all of our standard tumor panel protocols. It might also be noted that none of these agents possessed DN2 level activity (*see* footnote†, table 2) against the very refractory mouse colon 38 tumor model. Tumor model improvement is an area of applied research which undergoes continuous investigation in the NCI new drug program. In addition to the development of new in vivo models, efforts to develop several new in vitro models including the human tumor colony-forming assay are underway (3).

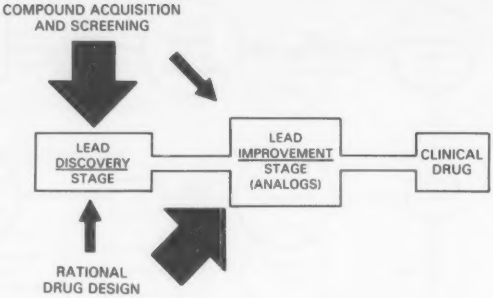

COMPOUND ACQUISITION AND SCREENING
LEAD DISCOVERY STAGE
LEAD IMPROVEMENT STAGE (ANALOGS)
CLINICAL DRUG
RATIONAL DRUG DESIGN

FIGURE 5.—Role of screening vs rational drug design in the drug discovery phase.

b. Compound selection.—Compound selection comprises the second major screening variable. Both synthetic compounds (4) and pure natural products (5,6) are acquired for testing. Figure 6 is a schematic representation showing how the compound selection and the biologic model variables are interrelated. No matter whether a compound is a potential new lead or an analog, it first enters the NCI in vivo P388 leukemia pre-

TABLE 1.—Evolution of NCI in vivo screening models

Year	Screening model
1955	Sarcoma 180, L1210 leukemia, Carcinoma 755
1960	L1210 + 2 models from a pool of 21
1965	L1210 + Walker 256 carcinosarcoma
1968	L1210 for synthetics; L1210 and P388 leukemia for natural products
1972	L1210 for synthetics; P388 for natural products; B16 melanoma and Lewis lung carcinoma for special testing
1975	P388 prescreen; screening of actives against a panel of mouse tumors ($CD8F_1$ mammary, colon 38, and Lewis lung) and human tumor xenografts (mammary, colon, and lung) of similar histologic types; L1210 and B16
1982	P388 prescreen; mammary xenograft, B16, L1210, M5076 tumor; additional testing against colon 38 and $CD8F_1$ tumors, as warranted

TABLE 2.—Performance of standard drugs in the 1975 NCI screen

Drug	Tumor panel systems with DN2 level activity*,†
Cisplatin	B16, CD, L1210, LL, CX, MX
Carmustine	B16, CD, L1210, LL, LX, MX
Melphalan	B16, CD, L1210, LX, CX, MX
Mitomycin	B16, CD, L1210, LX, CX, MX
Cyclophosphamide	B16, CD, L1210, LL, MX
Doxorubicin	B16, CD, L1210, LX, MX
Vincristine	B16, LX, MX
Cytarabine	L1210, LL, MX
6-Mercaptopurine	L1210
Methotrexate	L1210
Bleomycin	None

*B16 = B16 melanoma; CD = $CD8F_1$ mouse mammary; L1210 = L1210 leukemia; LL = Lewis lung mouse; CX = colon xenograft; LX = lung xenograft; and MX = mammary xenograft.

†All drugs also possess at least minimum P388 prescreen activity. DN2 level activity is defined as activity meeting or exceeding a standard value established for each model which would qualify a compound to be considered as a candidate for clinical trial.

screen (2). Active compounds may then pass into the tumor panel, and some of these are subsequently identified for further evaluation prior to selection as clinical candidates (figs 3 and 4). While NCI screening models have historically emphasized the selection of cytotoxic agents, a "P388 by-pass" mechanism exists which encourages evaluation of noncytotoxic materials such as cellular differentiating agents when there is a sound biochemical reason to do so.

Does the NCI method of compound acquisition lead to a "random screening" process? The answer is no—NCI compound acquisition is very selective from among the compounds offered by suppliers. Random screening can be defined as the testing of any compound that has not been tested previously. To begin with, the DTP feels that there are numerous classes of compounds which have been adequately tested for antitumor leads (table 3). Members of these classes are usually not tested unless there is a biologic rationale which suggests that a compound has unique properties relative to other members of its class. All of the compounds which are offered to the NCI for testing face a vigorous challenge prior to selection for screening (4). Figure 7 shows the selective acquisition process used to choose the compounds which are to be screened. An acquisition contractor working closely with DTP supplies the NCI with the structures of about 40,000 available compounds each year, in order to identify at least 20,000 compounds which the NCI has not already tested. First, a computer program identifies the duplicates. The structures of the remaining 20,000 new compounds are analyzed by two additional computer programs (7). One program rates the structural novelty of each new compound relative to the 370,000 compounds which the NCI already has tested. The other program is specially designed to predict P388 prescreen activity based on the structural characteristics of previously tested compounds. The resulting data are then analyzed by NCI medicinal chemists (PhD) who make the final selection of the 10,000 compounds to be screened, with special emphasis placed on novelty of structure.

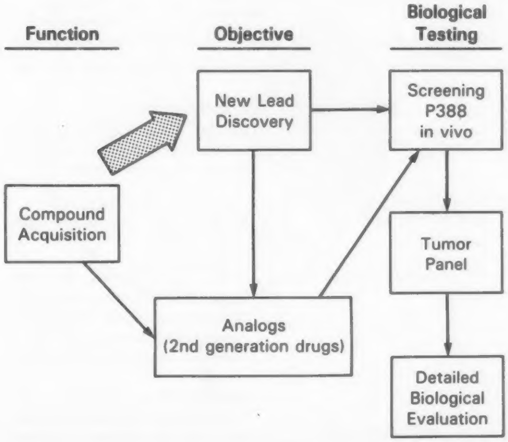


FIGURE 6.—Interrelationship of compound acquisition and testing.

Preclinical Development Phase

The NCI new drug program is organized to efficiently accomplish the work required to develop new leads and analogs to clinical trial. Approximately equal amounts of DTP resources are expended on the drug discovery and preclinical development phases (figs 1 and 2). Development requires functions not necessary in the discovery phase. These include special biologic testing, large-scale preparation, the development and production of clinical formulations, and, finally, toxicity studies.

Biologic testing.—Route and schedule dependency studies are carried out in tumored mice in order to provide benchmark information for subsequent formulation studies, toxicology tests, and clinical trials (2). Preliminary toxicity tests are also performed using nontumored mice.

TABLE 3.—Acquisition limitations

Category	Compound classes
A*	Nitrogen mustards Aziridines Nitrosoureas Triazenes (dacarbazine analogs) Epoxides
B†	Anthracyclines Antifols Alkylating agents in general Platinums

*Classes not generally accepted for screening when offered by suppliers.

†Classes occasionally accepted for screening but not actively acquired.

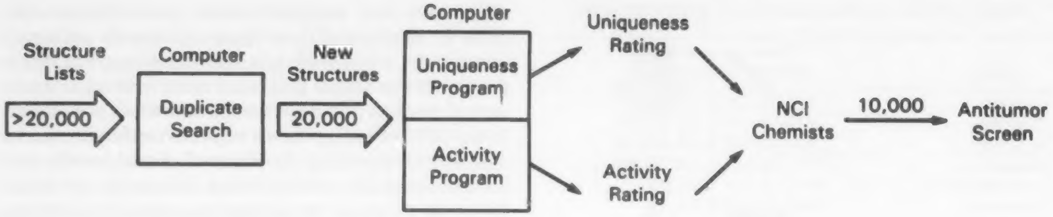


FIGURE 7.—Compound acquisition selection process.

Large-scale production.—Many of the compounds chosen as clinical candidates previously have been prepared only on a very small scale. However, multiple kilograms of a compound may be required to complete toxicity testing and clinical studies. Significant development work often is required to scale up the production of both synthetic agents and natural products. Feasibility studies for large-scale production under FDA-regulated conditions for clinical products are an important part of the preclinical development process.

Formulation development.—Following the discovery of a new lead, the possibilities of formulation for human administration are considered. Since most antitumor agents are administered by iv injection, the new drugs must be sufficiently soluble and stable in acceptable vehicle delivery systems. Only a very limited number of drug delivery vehicles are suitable for human administration (8). These vehicles normally contain > 80% water, and many of the new lead compounds, whose initial activity might have been determined using a drug suspension, are very water-insoluble. Additional problems occur when solutions of these active compounds are found to be unstable. The use of salts, cosolvents, surfactants, lipid emulsions, and prodrugs is among the techniques and approaches used to solve difficult formulation problems. Strict physical and chemical analytic criteria are applied to formulated products. It also must be demonstrated that the formulation retains antitumor activity. The DTP manufactures about one million injectable units each year for NCI-sponsored clinical trials.

Toxicology.—The feasibility of large-scale production, as well as formulation development, is always completed before a decision is made to start the toxicologic evaluation of a new drug (DN2B, fig 4). This is because the cost of a preclinical toxicology study currently exceeds $325,000/compound. Mouse lethality and dog toxicity studies are performed according to DTP-designed protocols acceptable to the FDA (9). Studies determine a safe starting dose for humans and specific organ toxicity in dogs, and establish the reversibility of the toxicity observed. Toxicity investigations complete the preclinical aspects of the NCI new drug process.

Clinical Phase

Following a successful toxicity study and a favorable DN Committee review (figs 3 and 4), all the preclinical data developed by the DTP are made available to the Cancer Therapy Evaluation Program. This DCT Program, which is responsible for the majority of NCI-sponsored clinical trials, assembles the required information and, as the drug sponsor, files an INDA with the FDA.

ANTICANCER DRUG ORIGINS

The DTP preclinical program has four major benchmarks which can be used to measure progress in the discovery and development of new anticancer drugs. These steps are shown in table 4. In order to assess the productivity aspects of the NCI new drug research program, it is useful to examine the origins of the compounds listed as statistics in table 4. Also of interest are the origins of those drugs which have become clinically established, commercially available, anticancer drugs.

Origin of Prescreen Compounds

Historical data indicate that 87% of the approximately 10,000 compounds tested by the NCI each year are ob-

TABLE 4.—New drug program statistics

A. Prescreen (P388 leukemia)	10,000 new compounds tested/yr Approximately 350 actives
B. Screen (tumor panel: L1210; B16; M5076; mammary xenograft)	250 compounds tested/yr Approximately 20 actives (DN criteria)
C. DN authorized further development	DN2A, approximately 10 compounds/yr selected as potential clinical candidates (large-scale production, formulation development, special biologic evaluation) DN2B, 7-9 compounds/yr put through NCI toxicology protocol
D. DCT INDA	DN3, 5-8 compounds/yr put into clinical trial

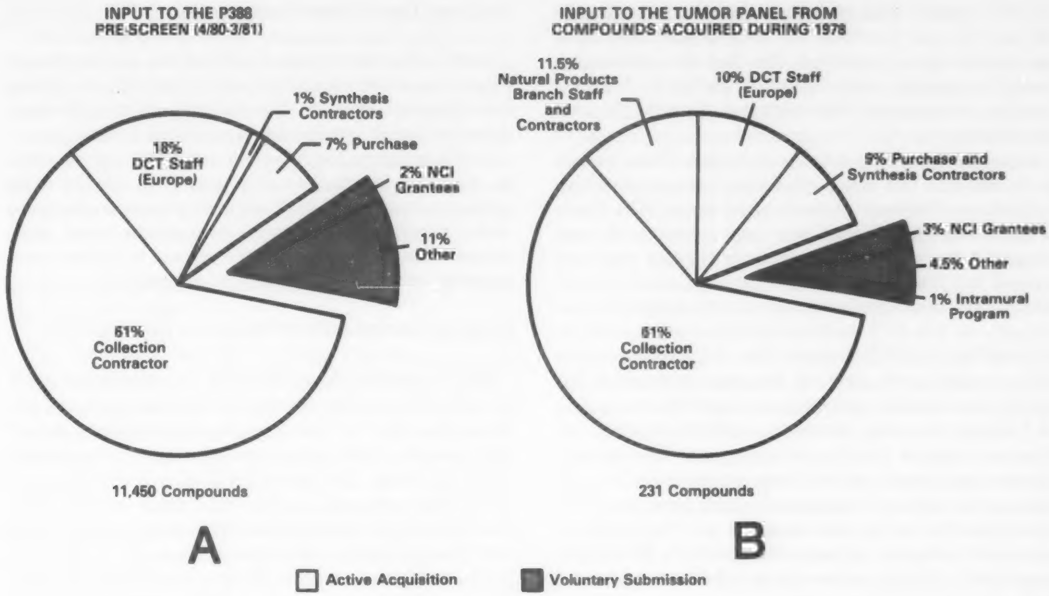


FIGURE 8.—Compound sources—(A) P388 prescreen Apr 80-Mar 81; (B) tumor panel for compounds acquired during 1978.

tained through an active acquisition process. Active acquisition compounds are defined as those obtained as a result of a direct request by DTP staff or its acquisition contractor. Most of these compounds are obtained as gifts from chemical and pharmaceutical companies in return for the antitumor test results. The remaining 13% of the compounds acquired are obtained through unsolicited, voluntary submissions from worldwide donors. Fig-

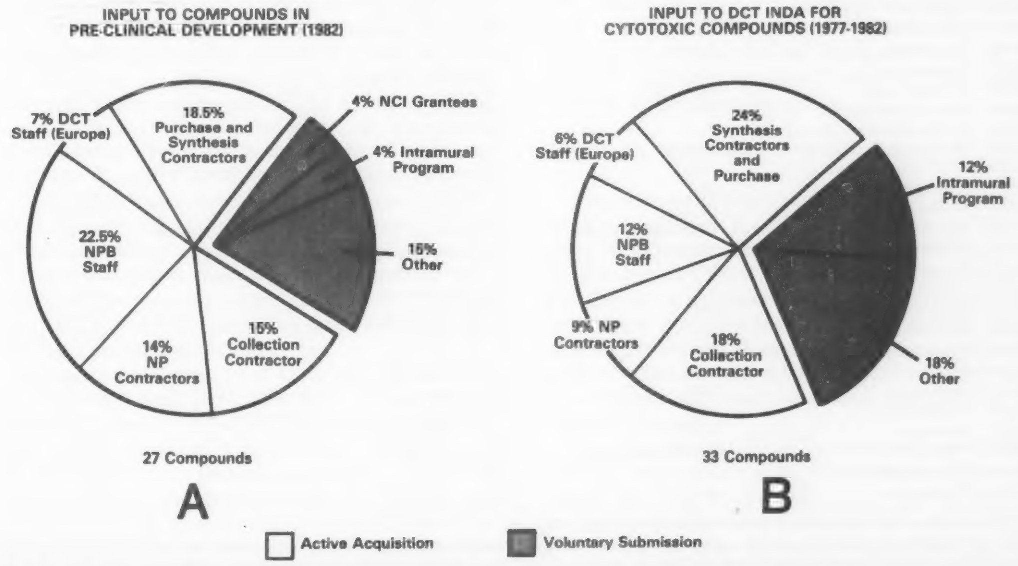


FIGURE 9.—Compound sources—(A) compounds in preclinical development on Jan 1, 1983; (B) DCT INDA for cytotoxic compounds 1977-1982.

ure 8A expands these prescreen statistics while figures 8B and 9A and B extend the data to the other three benchmarks given in table 4. The four time periods selected for analysis were chosen on the basis of reliable available data and are representative of recent program productivity.

Figure 8A shows input to the prescreen. This pie chart is divided into two major acquisition categories—active acquisition (87%) and voluntary submission (13%). These categories are subdivided into major sources of compounds. A few comments might be instructive about the sources. (*a*) The DTP supports a contract for the collection of compounds for antitumor testing (collection contractor). (*b*) The DCT maintains a liaison office in Brussels staffed by a PhD chemist. Part of the duties of this office include acquisition of European compounds for testing. (*c*) The NCI currently has very little in the way of funding for drug discovery synthesis contractors. However, because the drug discovery and development process sometimes requires many years, there are still some compounds in development which came from previously funded synthesis contractors. (*d*) The voluntary submission category includes NCI grantees, DCT intramural staff, and any other sources which are not identified as active acquisition sources.

Origin of Tumor Panel Compounds

Input to the tumor panel (fig 8B) shows an even greater reliance on active acquisition, with a 91 to 9 ratio relative to voluntary submission. Natural products, as actively acquired materials, are identified separately here.

In this category, the collection contractor, coincidentally, makes an identical contribution (61%) relative to its prescreen input. DCT staff and synthesis contractors also make significant contributions to tumor panel compounds. The results of the first 7 years of the tumor panel experiment have been summarized recently (10).

Origin of Compounds in Preclinical Development

Only the best of the compounds from the tumor panel are chosen for further preclinical development by the DN Committee (fig 3). This category is particularly important because these compounds are targeted for clinical trial if DN steps DN2A and DN2B (figs 3 and 4) are accomplished successfully. The structures of the compounds currently in preclinical development are available (11). Compounds in these two steps as of January 1, 1983, are given in table 5 along with detailed information regarding how the compounds were acquired and the

TABLE 5.—Compounds in preclinical development (DN status 2A or 2B on Jan 1, 1983)

NSC No.	Compound	Antitumor discovery institution*	Submission mode	Source	NCI preclinical role
127755	Dihydrotriazine	NCI	Voluntary	B. R. Baker	Discovery
156492D	Discreet	NCI	Active acquisition	—	Discovery
226080	Rapamycin	NCI	Active acquisition	Ayerst	Discovery
234714	Aphidicolin	NCI	Active acquisition	ICI†	Discovery
237020	Largomycin	Tanabe	Active acquisition	Tanabe	Significant
253272	Caracemide	NCI	Active acquisition	Dow	Discovery
261726	3-Deazaguanine	ICN, USC†	Voluntary	ICN†	Little
267213	Sulfonyl hydrazone	NCI	NCI contract	SRI†	Discovery
268242	*N*-Dibenzyldaunorubicin	NCI	NCI contract	SRI	Discovery
269148	Menogarol	NCI	NCI contract	Upjohn	Discovery
271674	DACH (cisplatin analog)	NCI	NCI contract	Veterans Administration	Discovery
278214	Isopropyl Pyrrolizine	NCI	Voluntary	SUNY	Discovery
281272	Arabinosyl-5-azacytosine	NCI	Intramural	LMCB†	Discovery
283162	Trimethyl trimethylolmelamine	Chester Beatty Institute	Voluntary	Chester Beatty Institute	Significant
284356	Mitindomide	NCI	Active acquisition	Gulf	Discovery
293015	Flavone analog	NCI	Active acquisition	Lipha	Discovery
303861D	Discreet	NCI	Active acquisition	—	Discovery
308847	Benzoisoquinolinedione	NCI	Active acquisition	L. MADE†	Discovery
314055	Radiosensitizer SR 2555	NCI	NCI contract	SRI	Discovery
322921	Bisbenzimidazole	NCI	Active acquisition	Purchase	Discovery
325014	Bactobolin	IMC	Active acquisition	IMC†	Significant
325319	Didemnin B	NCI	NCI contract	University of Oklahoma	Discovery
328426	Phyllanthoside	NCI	NCI contract	University of Virginia	Discovery
330500	Macbecin II	Takeda	Active acquisition	Takeda	Significant
333856	Tetrocarcin A	Kyowa Hakko Kogyo	Active acquisition	Kyowa Hakko Kogyo	Significant
339638	Pyranone phosphate	NCI	NCI contract	Warner-Lambert	Discovery
352122	Trimetrexate	NCI	Voluntary	Warner-Lambert	Discovery

*NCI-funded discovery (excluding grants) shows NCI as discovery institution.

†ICI = Imperial Chemical Industries; ICN = International Chemicals and Nuclear Corp; USC = University of Southern California; SRI = SRI International; SUNY = State University of New York, Buffalo (*W. Anderson*); LMCB = Laboratory of Medicinal Chemistry and Biology, DTP, DCT, NCI; L. MADE = Laboratories MADE, S. A. (Spain); IMC = Institute of Microbial Chemistry, Japan (*H. Umezawa*).

role of the NCI in their discovery and development.

The sources are analyzed somewhat differently in figure 9A. Active acquisition made up 77% of the 27 compounds in preclinical development at the beginning of 1983. The DTP acquisition and screening program was responsible for discovering the antitumor activity of 21 of 27 compounds in this category. In addition, significant DTP preclinical development resources are being expended in testing, formulation, scale-up, and toxicology studies for five of the remaining six compounds.

Origins of Cytotoxic Compounds With INDA Filed During 1977-1982

The NCI filed 42 INDA during the 6-year period, 1977-1982 (table 6). Four of these filings (amygdalin, tetrahydrocannabinol, heroin, and levonantradol) were for compounds without antitumor activity—analgesics, antiemetics, or miscellaneous compounds not associated with the NCI new drug program. Five additional INDA were filed for radiosensitizing or radioprotecting agents. While developed with DTP resources, the selection of these five compounds was based on studies by the Radiation Research Program, DCT. This leaves 33 compounds which can be classified as cytotoxic agents and products of the DTP new drug program. These are the compounds whose origins are analyzed in figure 9B and table 7.

Active DTP acquisition accounted for 23 of 33, or 69%, of INDA filings during this period. Seven of the 23 filings were natural products while 16 were synthetic agents. The decrease in the collection contractor contribution from 60% to the 15% level is a natural consequence of emphasis on the collection of new structural types rather than analogs. There is a significantly higher probability of synthesizing an analog which will achieve a clinical trial than there is of discovering a completely new structural type which achieves that status. Synthesis contractors and voluntary contributors historically have emphasized lead improvement (ie, analogs) rather than new lead discovery. Clearly, the analogs prepared were chosen for good reasons, but nevertheless, analogs are not new leads. Since the major objective of the screening program is the development of new leads, the job of the acquisition contractor is to make compounds available which have potential for this purpose. That this is occurring is borne out if the INDA data are reanalyzed in terms of new leads and analogs (table 8).

New leads account for 19 of 33, or 58%, of the INDA which the DCT filed during this 6-year period. The five compounds acquired by the collection contractor (levamisole, deoxycoformycin, mitoxantrone, acodazole, and tiazofurin) all fall into the new lead category. While these data may indicate that screening is not an efficient process, there is really no reason to expect that it should be, given its objective: new lead discovery. All of the compounds shown in table 7, with the exception of the ten voluntary submissions, are attributable to the DCT active acquisition program.

Examining the ten voluntary submissions in more detail (table 7), all four of the voluntary submissions classed as analogs came from the DCT intramural program. These compounds are diaziquone (AZQ), ketotrexate (also known as 5-methyltetrahydrohomofolate), dihydro-5-azacytidine, and spiromustine. All are products of rational drug design and, therefore, are classed as lead improvement rather than new leads. Although listed as voluntary submissions, these four compounds could have been attributed to active acquisition since the DTP intramural program always has interacted very closely with the extramural acquisition effort.

Spirogermanium, bisantrene, and IMPY are three of the six new leads obtained as voluntary submissions. Two compounds (DON and *N*-methylformamide) were submitted during the 1950s and it was difficult to trace their history. They arbitrarily were called voluntary submissions. The sixth compound, amsacrine (AMSA), is a clinically valuable voluntary submission from Dr. Bruce Cain (deceased) of New Zealand. The activity of AMSA was discovered in Dr. Cain's screening program. This compound is one of > 800 in this series which he synthesized and screened using the L1210 tumor line supplied to him by the DTP. It should be mentioned that the cost in DTP resources, to develop a voluntary submission such as AMSA to clinical trial, is essentially identical to the cost of readying a compound discovered within the NCI screening program.

Summarizing the INDA compound data from the 33 cytotoxic compounds filed during 1977-1982, it can be seen that the NCI new drug program discovered the antitumor activity of 73% of these compounds (table 7) and 100% of the compounds filed during 1982 (table 6). Active DTP acquisition accounted for 68% of the new leads and 71% of the analogs (table 8).

Origins of Commercially Available Drugs

Those compounds which prove to be useful during the INDA stage (phase I, II, and III clinical trials) are the subject of New Drug Applications (NDA) filed with the FDA. Drugs achieving NDA status become commercially available. The new drug process, from discovery through NDA approval, is a lengthy one. Table 9 indicates a typical time frame for an anticancer drug. While examples could be found with time periods significantly shorter or longer than 14 years, this figure serves as a rough guide to the time period required.

There are presently 32 nonhormonal anticancer drugs commercially available in the United States. These compounds have been well-reviewed with regard to their discovery, development, and mechanism of action (12-15). It would appear from their antitumor publication dates that at least one-half of these compounds were discov-

TABLE 6.—NCI INDA filings, 1977-1982

Year	NSC No.	Compound	Antitumor discovery institution*	Submission mode	Source	NCI preclinical role
1977	51143	IMPY	Ciba	Voluntary	Ciba	Significant
	132319	Indicine-*N*-oxide	NCI	NCI contract	Pfizer	Discovery
	165563	Bruceantin	NCI	NCI contract	University of Virginia	Discovery
	177023	Levamisole	Janssen	Active acquisition	Lederle	Little
	224131	PALA	NCI	Active acquisition	Stanford University	Discovery
	249992	Amsacrine	ADCS‡	Voluntary	ADCS	Significant
	261037	Oral misonidazole†	Roche	Voluntary	Roche	Little
1978	15780	Amygdalin	Not applicable	—	—	—
	21548	Thymidine	NCI	NCI contract	Stehlin	Discovery
	95466	PCNU	NCI	NCI contract	SoRI‡	Discovery
	118742	Pentamethylmelamine	NCI	NCI contract	Chester Beatty Institute	Significant
	134454	Tetrahydrocannabinol	Not applicable	—	—	—
	153353	Alanosine	MSKCC‡	Active acquisition	LePetit	Significant
	169780	ICRF-187	ICRF‡	Active acquisition	ICRF	Significant
	302357	Heroin	Not applicable	—	—	—
1979	7365	DON	NCI§	Voluntary	SoRI	Discovery
	163501	Acivicin	NCI	NCI contract	Upjohn	Discovery
	182986	Diaziquone	NCI	Intramural	LMCB	Discovery
	208734	Aclacinomycin	IMC	Active acquisition	IMC	Little
	218321	Deoxycoformycin	NCI	Active acquisition	Warner-Lambert	Discovery
	261037	Iv misonidazole†	Roche	Voluntary	Roche	None
	301739	Mitoxantrone	NCI	Active acquisition	MRI, Allied	Discovery

1980	139490	Ketotrexate	NCI	Intramural	Drug Evaluation Branch	Discovery
	261036	Desmethylmisonidazole†	Roche	Voluntary	Roche	None
	296961	WR-2721†	Walter Reed	Active acquisition	Walter Reed	Little
	331615	Levonantradol	Not applicable	—	—	—
	404241	Arabinosyladenine	NCI	NCI contract	SoRI, MDA‡	Discovery
1981	40774	6-Methylmercaptopurine riboside	NCI	NCI contract	MSKCC, SoRI	Discovery
	141633	Homoharringtonine	NCI	Active acquisition	USDA‡	Discovery
	192965	Spirogermanium	Georgetown University	Voluntary	Georgetown University	Little
	241240	CBDCA (cisplatin analog)	NCI	NCI contract	Chester Beatty Institute	Significant
	264880	Dihydro-5-azacytidine	NCI	Intramural	LMCB	Discovery
	280594	Tricyclic nucleotide	NCI	NCI contract	University of Utah	Discovery
	296934	Teroxirone	NCI	Active acquisition	Henkel	Discovery
	337766	Bisantrene	Lederle	Voluntary	Lederle	None
	526417	Echinomycin	MSKCC	Active acquisition	Shionogi	Significant
1982	3051	*N*-Methylformamide	NCI§	Voluntary	SoRI	Discovery
	172112	Spiromustine	NCI	Intramural	LMCB	Discovery
	286193	Tiazofurin	NCI	Active acquisition	ICN	Discovery
	301467	Radiosensitizer SR2508†	NCI	NCI contract	SRI	Discovery
	305884	Acodazole HCl	NCI	Active acquisition	Norwich	Discovery
	312887	Fludarabine-5′-phosphate	NCI	NCI contract	SoRI	Discovery

---

[*] NCI-funded discovery (excluding grants) shows NCI as discovery institution.

†Radiosensitizer or radioprotector. RRP compounds defined here as noncytotoxic compounds.

‡ADCS = Auckland Division Cancer Society of New Zealand (*B. Cain*); MSKCC = Memorial Sloan-Kettering Cancer Center; ICRF = Imperial Cancer Research Foundation; SoRI = Southern Research Institute; MRI = Midwest Research Institute; MDA = M. D. Anderson Hospital and Tumor Institute; USDA = US Department of Agriculture.

§INDA based on data from new NCI tumor panel models.

TABLE 7.—Analysis of the 33 NCI INDA for cytotoxic agents (1977-1982)

	No. (% of grand total)
Source analysis	
Active acquisition (23)	
Synthetics	
Collection contractor	5 (15)
DCT staff (Europe)	2 (6)
Purchase, synthesis contractors	9 (27)
Subtotal	16 (48)
Natural products	
DCT staff	4 (12)
Fermentation contractors	1 (3)
Plant and animal contractors	2 (6)
Subtotal	7 (21)
Total active acquisitions	23 (69)
Voluntary submissions (6)	
NCI grantees	0 (0)
DCT intramural program	4 (12)
Other	6 (18)
Total voluntary submissions	10 (30)
Grand total	33 (99)
Discovery of antitumor activity	
NCI	24 (73)
Other	9 (27)
Total	33 (100)

ered prior to the inception of the NCI new drug program in 1955. These drugs are shown in table 10. During this early period, the discovery of the antitumor activity for more than one-half of these drugs can be attributed to pharmaceutical companies. The time required for completion of the drug development process cannot be determined from the antitumor publication and NDA approval dates as shown in table 10. INDA filings were not required at that time and patent considerations undoubtedly played a role in determining the period between publication and NDA approval. The dates shown in tables 10 and 11 refer to the publication of antitumor discovery, not compound discovery.

TABLE 9.—Typical time frame for DCT new drug operations

Step No.	Operation	Approximate time to completion (cumulative)
1	Prescreen activity discovered and confirmed	6 mos
2	Tumor panel completed and activity confirmed	2 yrs
3	Pass DN2A (start formulation development, large-scale production)	3 yrs
4	Pass DN2B (start toxicology)	5 yrs
5	INDA filed	6 yrs
6	Phase I clinical studies completed (safety)	7 yrs
7	Phase II clinical studies completed (effectiveness)	9 yrs
8	Phase III clinical studies completed (comparison)	12 yrs
9	NDA approved	14 yrs

Table 11 shows drugs whose antitumor publication dates indicate that they were discovered since the inception of the NCI new drug program. The NCI has played a discovery role in eight of 16 of these NDA drugs. Of these eight, only o,p-DDD is independent of the DCT screening program. The antitumor effect of this compound was discovered by NCI intramural scientists in the NIH Clinical Center. Relative to the other NCI-discovered compounds, hydroxyurea and pipobroman were off-the-shelf compounds collected in the early days of the NCI screening program. Mithramycin and streptozocin were discovered under NCI-funded fermentation contracts. The nitrosoureas, carmustine (BCNU) and lomustine (CCNU), and the imidazolecarboxamide, dacarbazine (DTIC), were synthesized by the Southern Research Institute under a DCT contract. These nitrosoureas were analogs of methylnitrosoguanidine, a compound whose activity was discovered in the NCI screening program. Even though the NCI did not discover the initial activity of cytarabine and cis-

TABLE 8.—Sources of new leads: 33 NCI INDA filed for cytotoxic agents (1977-1982)

Source	No. of— New leads	Analogs
Synthetic compounds		
Collection contractor	5	0
DCT staff (Europe)	0	2
Purchase, other	1	1
Synthesis contractors	1	6
Voluntary submissions	6	4
Natural products		
Natural Products Branch staff	3	1
Natural Products Branch contractors	3	0
Total	19	14

TABLE 10.—Origin of commercially available anticancer drugs* —drugs with publication dates which indicate discovery was made prior to start of the NCI new drug program in 1955

NSC No.	Drug	Antitumor discovery institution	Year of— Antitumor publication	NDA
762	Mechlorethamine	U.S. Army, Yale	1946	1949
740	Methotrexate	MSKCC, Lederle	1949	1953
9706	Triethylenemelamine	Lederle	1950	1953
750	Busulfan	Chester Beatty Institute	1951	1954
6396	Thiotepa	Lederle	1952	1959
755	6-Mercaptopurine	Wellcome, MSKCC	1953	1953
3088	Chlorambucil	Chester Beatty Institute	1953	1957
752	Thioguanine	Wellcome, MSKCC	1954	1966
8806	Melphalan	Chester Beatty Institute	1954	1964
3053	Dactinomycin	Children's Medical Center	1955	1964
26980	Mitomycin	Institute of Microbial Chemistry	1956	1974
19893	5-FU	University of Wisconsin	1957	1962
27640	FUDR	University of Wisconsin	1957	1970
49842	Vinblastine	University of Western Ontario	1958	1961
26271	Cyclophosphamide	Asta Werke	1958	1959
34462	Uracil mustard	Upjohn	1958	1962

* Nonhormonal drugs only.

TABLE 11.—Origin of commercially available anticancer drugs* —drugs with publication dates after 1958

NSC No.	Drug	Antitumor discovery institution	Source	Year of — Antitumor publication	NDA	NCI preclinical role†
38721	o,p-DDD	NCI	Intramural	1959	1970	Discovery
24559	Mithramycin	NCI	Pfizer	1960	1970	Discovery
63878	Cytarabine	Upjohn	Upjohn	1961	1969	Significant
45388	Dacarbazine	NCI	SoRI	1962	1975	Discovery
67574	Vincristine	Lilly	Lilly	1962	1963	None
25154	Pipobroman	NCI	Abbott	1962	1966	Discovery
85998	Streptozocin	NCI	Upjohn	1962	1982	Discovery
32065	Hydroxyurea	NCI	Squibb	1963	1967	Discovery
77213	Procarbazine	Roche	Roche	1963	1969	None
409962	Carmustine	NCI	SoRI	1963	1977	Discovery
109229	L-Asparaginase	Cornell	Cornell	1963	1978	Significant
82151	Daunorubicin	Farmitalia, Rhone Poulenc	Farmitalia	1963	1979	Little
125066	Bleomycin	IMC	IMC	1965	1973	Little
79037	Lomustine	NCI	SoRI	1966	1976	Discovery
123127	Doxorubicin	Farmitalia	Farmitalia	1968	1974	Little
119875	Cisplatin	Michigan State	Michigan State	1969	1978	Significant

* Nonhormonal drugs only.

†NCI had a major role in the clinical development of all these drugs.

platin in its program, the preclinical effort of the NCI was decisive in the development of these two drugs to clinical trial and eventual NDA status. It is unlikely that these two compounds would be available to the public today without this effort.

REFERENCES

1. DEVITA VT, OLIVERIO VT, MUGGIA FM, ET AL. The drug development and clinical trials program of the Division of Cancer Treatment, National Cancer Institute. Cancer Clin Trials 2:195-216, 1979.
2. VENDITTI JM. Preclinical drug development: rationale and methods. Semin Oncol 8:349-361, 1981.
3. ______. The National Cancer Institute antitumor drug discovery program, current and future perspectives: a commentary. Cancer Treat Rep 67:767-772, 1983.
4. NARAYANAN V. Strategy for the discovery and development of novel anticancer agents. *In* Structure-Activity Relationships in Anti-Tumour Agents (Reinhoudt DN, Connors TA, Pinedo HM, et al, eds). The Hague, Martinus Nijhoff, 1983, pp 5-22.
5. SUFFNESS M, and DOUROS J. Current status of the NCI plant and animal product program. J Nat Prod 45:1-14, 1982.
6. DOUROS J, and SUFFNESS N. New antitumor substances of natural origin. Cancer Treat Rev 8:63-87, 1981.
7. HODES L. Selection of molecular fragment features for structure-activity studies in antitumor screening. J Chem Inf Comput Sci 21:132-136, 1981.
8. DAVIGNON JP, and CRADOCK JC. The formulation of anticancer drugs. *In* Principles of Chemotherapy (Carter SK, and Hellman K,

eds). New York, McGraw-Hill, in press.

9. LOWE MC, and DAVIS RD. The current toxicology protocol of the National Cancer Institute. *In* Principles of Chemotherapy (Carter SK, and Hellman K, eds). New York, McGraw-Hill, in press.
10. VENDITTI JM, WESLEY RA, and PLOWMAN J. Current NCI pre-clinical antitumor screening in vivo. Results of tumor panel screening, 1976–1982, and future directions. *In* Advances in Pharmacology and Chemotherapy, New York, Academic Press, vol 20, in press.
11. LOMAX NR, and NARAYANAN VL. Chemical structures of interest to the Division of Cancer Treatment, vol III, 1983. (Available from Chief, Drug Synthesis & Chemistry Branch, DTP, DCT, NCI, NIH, Bethesda, MD 20205).
12. MONTGOMERY JA, JOHNSTON TP, and SHEALY YF. Drugs for neoplastic diseases. *In* Medicinal Chemistry, 3rd ed, part I (Burger A, ed). New York, Wiley, 1970, pp 680–783.
13. PRATT WB, and RUDDON RW. The Anticancer Drugs. New York, Oxford, 1979, pp 12–19.
14. Cancer, a Comprehensive Treatise. *In* Chemotherapy (Becker FF, ed). New York, Plenum Press, 1977, vol 5, pp 285–519.
15. Antineoplastic and Immunosuppressive Agents, part II (Sartorelli AC, and Johns DG, eds). New York, Springer-Verlag, 1975, pp 1–892.

Clinical Development of Anticancer Agents—A National Cancer Institute Perspective

Silvia Marsoni* and Robert Wittes[1]

Since the first report that a chemical could cause significant tumor shrinkage (1), the development of clinically useful antineoplastic drugs has grown from the preoccupation of a few investigators to a major international effort. Over the past 35 years or so, about 30 drugs have been defined as active in one or more tumor types. When used alone in patients with disseminated malignancy, these drugs cause reduction in bulk of measurable neoplasm in a significant percent of cases; for most tumor types, however, ample evidence of residual cancer usually persists and after a few months, tumor regrowth occurs. More striking successes have been achieved with combinations of drugs; as is well known, for several kinds of disseminated human cancers, a high frequency of clinical complete remissions, with substantial long-term disease-free survival rates, is now possible (2-5). For other cancers which may not be curable by chemotherapy once they have disseminated, combinations of drugs appear to result in a higher total remission rate and a greater prolongation of life than single drugs (6,7). Perhaps more significant in the long run is the apparent effect of chemotherapy when it is used as part of a planned multimodality effort (8,9).

By some perverse quirk of fate, chemotherapy seems to chiefly exert a major impact in rare tumors, while the common epithelial neoplasms of adulthood have thus far resisted satisfactory solutions. Therefore, the central problem of drug development, the identification of effective agents with reasonable therapeutic index, is as pertinent for oncology now as at any time in the past.

The idealized outlines of the successive steps in drug development are familiar to all oncologists. In phase I trials, we define a dose suitable for use in studies of the drug's activity across a spectrum of human tumors. Increasing awareness of the importance of patient- and disease-related parameters has effectively led to the replacement of the broad phase II trial with a series of disease-oriented activity studies. Having defined settings in which the new drug is active, investigators then proceed to compare the new treatment with standard therapy (phase III) and to further explore the drug's therapeutic potential in other ways, such as in combination with other agents or by alternate routes of administration.

Anyone familiar with the actual workings of this process over the past two decades knows that despite its successes, it has not functioned as systematically or efficiently as the above description might imply. In addition, many of the assumptions on which the process was based are in need of re-examination. Since there are no reliable laboratory predictors of efficacy for specific human cancers, drug development will continue to require extensive testing in human subjects, an endeavor that is never without ethical dilemmas, however thoughtfully it is carried out. In addition, because human cancers vary widely in sensitivity to anticancer drugs, the apparatus required to sustain the clinical trials effort is necessarily large and very expensive. For these reasons alone, another look at the drug development program of the National Cancer Institute (NCI) seems to be worthwhile.

Phase I

Phase I trials of antineoplastic compounds are conducted in patients with disseminated malignancies for whom standard treatment either does not exist or has proved ineffective. Drugs are given in a phase I trial with therapeutic intent; the main scientific goal is to define the qualitative and quantitative characteristics of the drug's acute toxicity, and in so doing, to determine a biologically active dose which is tolerable for every patient. The maximum tolerated dose (MTD) is usually higher in children than in adults (10), probably because of better organ function and possibly because of different pharmacokinetics (11,12). Also, since the MTD for patients with acute leukemia is often substantially higher than that for solid tumors, at least four

---

[1] Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Silvia Marsoni, MD, Drug Evaluation and Reporting Section, Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Landow Bldg, Rm 4C09, 7910 Woodmont Ave, National Institutes of Health, Bethesda, MD 20814.

phase I trials should be conducted for each drug. In practice, of course, three to four phase I trials testing different schedules of the drug are usually performed in adult patients with solid tumor alone, and trials in children do not start until substantial experience is accumulated from the adult trials.

The assumption underlying all phase I escalation procedures is that anticancer compounds must be given at or near the MTD; therefore, the job of a phase I trial is to define the highest dose that can be safely delivered to a patient, since this dose will also be the one that has the best chance of being active. This approach reflects the difficulties in establishing a clear-cut measurable endpoint for drug activity against cancer. Since induction of response is not usually an event that is immediately recognizable, attainment of toxicity is the only assurance that, if a response is not obtained, at least a biologically active dose was delivered. Underlying this assumption is the more fundamental one that the dose-response curve for human cancers is monotonically increasing throughout the range of tolerated doses and is to the right of the dose-toxicity curve. Needless to say, the details of this assumption have not been generally verified for antitumor agents, chiefly because rigorously defining the shape of a clinical dose-response curve is a laborious task, requiring a large number of patients treated at each of several dose levels. Where the relationship between dose and response has been examined, however, most of the data are at least consistent with the conclusion that the higher the administered dose, the more probable an antitumor effect (13-17) or the longer the duration of remission (18). On the other hand, recent trials in small cell lung cancer suggest that the probability of response does not continue to increase linearly as the dose approaches the MTD (19).

For most of the clinically useful compounds, the bone marrow is dose-limiting. The dose-toxicity curve for myelosuppression is quite reproducible, and the status of marrow reserve is the major source of interpatient variability. Generally, treatment of six to ten patients at or near the MTD is sufficient to establish a safe phase II dose when myelosuppression is the dose-limiting toxicity.

However, major problems may arise when other toxic effects which are less easily quantifiable are dose-limiting. For example, in a phase I study of escalating doses of carmustine with autologous bone marrow support (20), major organ toxicity (liver, central nervous system, and lung) surfaced abruptly at a dose of 1500 mg/m^2. Because of the sudden appearance of these side effects in the escalation scheme and the long interval from the beginning of treatment to onset of toxicity (6-9 weeks), the overall mortality rate for patients entered at a dose of $\geq$ 1500 mg/m^2 was approximately 30%.

Experience suggests that whenever a drug has dose-limiting side effects other than myelosuppression, its transition into phase II has often been compromised. An analysis of 31 drugs entered in phase I evaluation by the NCI shows that whenever the drug had myelosuppression alone as the dose-limiting toxicity, it had a high probability of undergoing full phase II study; however, when other organ toxicity was dose-limiting, only 25% of the drugs proceeded to full phase II study (table 1). Evaluation of the remainder of drugs was restricted by either the NCI or lack of investigator interest. The main reason for these difficulties relates largely to the uncertainty regarding reversibility of acute major organ damage. In addition, even if organ damage should turn out to be reversible, medical support during periods of severe organ failure is either extremely intensive and costly (kidney, CNS) or technically unsatisfactory (liver), and is not seen as feasible or justifiable by most investigators in the context of a clinical experiment.

TABLE 1.—Phase II evaluation as a function of the dose-limiting toxicity of 31 cytotoxic compounds (1975-1982)

Phase II evaluation	Dose-limiting toxicity		
	Myelosuppression[*]	Myelosuppression and organ toxicity[†]	Organ toxicity[‡]
Full	10	3	3
Restricted	0	3	4
Dropped			
Toxicity	0	0	4
No interest	1	1	1
No drug supply	1	0	0
Total	12	7	12

[*] Bisantrene, diaziquone, aclarubicin (aclacinomycin), mitoxantrone, PCNU, amsacrine, zorubicin, chlorozotocin, carboplatin, ICRF 187, 5-methyltetrahydrohomofolate, and 3-deazauridine.

[†] Acivicin, maytansine, anguidine, teroxirone, dihydro-5-azacitidine, indicine *N*-oxide, and DON.

[‡] Pyrazofurin, L-alanosine, homoharringtonine, TCN-P, *N*-methylformamide, pentostatin, hycanthone, dichloroallyl lawsone, pyrazole, aminothiadiazole, bruceantin, and spirogermanium.

This dilemma appears to have no easy solutions. One alternative might be to utilize data from pharmacologic studies to define the relationship between dose, plasma level, tissue level, and clinical activity. For example, in the case of pentostatin, knowledge concerning the amount of drug needed to abolish activity of the target enzyme adenosine deaminase has helped to establish a phase II dose independent of the attainment of clinical toxicity. Unfortunately, however, this is an exceptional situation and, under most circumstances, specific intracellular targets of drug action either have not been identified or are not so susceptible to analysis.

A somewhat more empiric approach is exemplified by the current plans for developing *N*-methylformamide, a drug which had been introduced into the clinic in the 1950s and was subsequently dropped while in phase I because of hepatotoxicity (21). Interest in the drug has recently been revived because of its activity against human tumor xenografts (22) and its capacity to induce differentiation in vitro (23). Phase I trials in both the United Kingdom and the US confirm that, at a dose of 1000 mg/m^2, the reversible hepatotoxicity of *N*-methylformamide is dose-limiting and myelosuppression is completely absent.[2,3] This dose has been defined as the MTD, at which phase II trials have just begun. If activity is observed in any tumor type, a repeat phase II study in one or more susceptible tumors will be performed at a level immediately below the MTD. This procedure will define whether the attainment of toxicity is necessary for activity.

Phase II

In a phase II trial, the main goal is to assess the activity of the drug in a variety of disseminated malignancies and to further define the patterns of acute toxicity in patients who are homogeneous in diagnosis and in better general medical condition than patients in phase I. Since large numbers of patients are treated during phase II, rarer acute toxic effects often surface for the first time (24). Also, since cumulative drug doses may be appreciable in responding or stable patients, phase II provides an appropriate setting for initial assessment of chronic toxic effects.

Several vexing problems are inherent in this process. In the first place, since patient numbers and resources are finite, it is impossible to explore the activity of each drug in each tumor type. A method must be found to focus the effort of drug development in a way that will minimize the chance of overlooking active agents. Accordingly, the NCI decided to evaluate all experimental drugs in selected types of cancer. The NCI Human Tumor Panel was created in 1975 and included lung, colon, and breast carcinomas, and lymphoma, leukemia, and melanoma. The original intention was to match tumors in the human panel with those in the preclinical panel, thereby providing information for the validation of the preclinical screening program. In addition, these classes of human cancer represent the two extremes of chemotherapy sensitivity and might be expected to exhibit both high sensitivity and high selectivity. Finally, the inclusion of the most common causes of cancer deaths (breast, colon, and lung cancers) permits the study of large numbers of patients and assures that results will have immediate implications for the treatment of prevalent cancers. Needless to say, evaluation of individual drugs is also carried out in tumors other than those in the panel, particularly if there is a specific reason to do so. For example, diaziquone was chemically designed to cross the blood-brain barrier and therefore has been extensively evaluated in brain tumors with encouraging results.

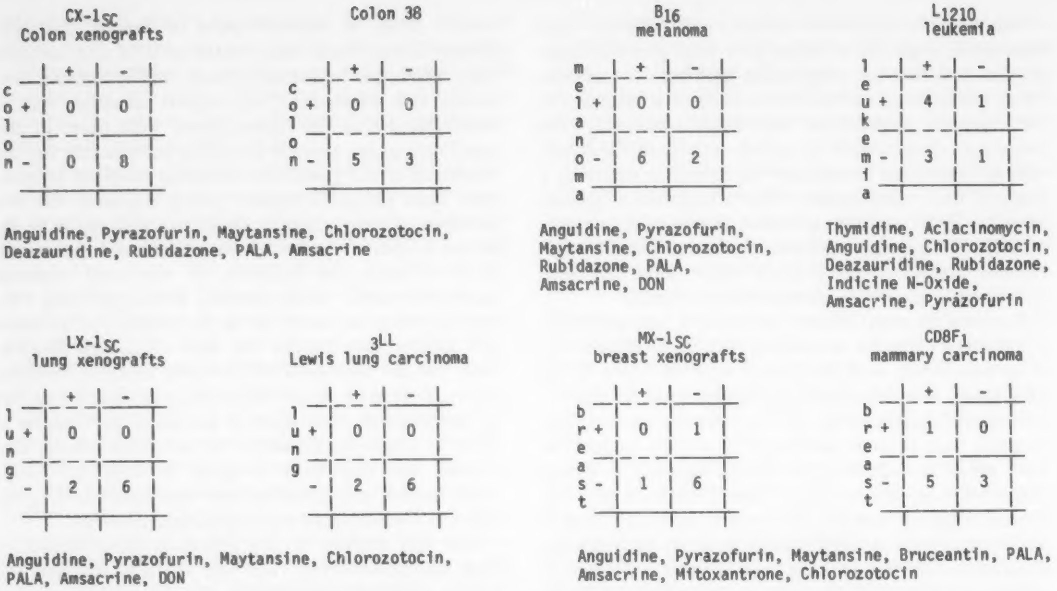
How has activity in the prelinical panel correlated with clinical activity? Thus far, we have analyzed the results with 13 experimental drugs for which clinical and experimental data are available. The correlation of activity in each model tumor system with activity in the corresponding human cancer is shown in figure 1. Prediction of true-negative results (resistance) seems fairly reliable across most of the rodent and xenograft systems. On the other hand, the probability of predicting true-positive results (sensitivity) is very low. Because of the small number of active drugs in humans for which complete data are available, no definitive conclusions can be drawn. However, even if the preclinical panel should not turn out to be an accurate predictor of response in individual tumor types, overall activity in prelinical screening may still serve as a general predictor of activity in at least one human cancer. The aggregated data are, in fact, consistent with this notion. This has obviously been the general premise on which antitumor screening programs have operated for years. Its validity has been widely assumed but has not been subjected to direct test, since drugs are not brought to the clinic if screening data are not positive. An assessment of the validity of the assumption will be afforded by the use of the human tumor stem cell assay as a screening tool. The plans are to bring selected compounds which are positive in the human tumor stem cell assay to clinical trial, even if they are negative in the P388 prescreen (26). Obviously, more data are needed to determine the ultimate usefulness of the panel.

How has the human panel fared as a predictor of clinical efficacy in human tumors other than those of the panel? Since 1971, 62 cytotoxic agents have been introduced into clinical trials under the sponsorship of

---

[2] McVie JG, ten Bokkel Huinink WW, Simonetti G, et al. Phase I trial of *N*-methylformamide (NSC 3051) (NMF). Manuscript submitted to *Cancer Treatment Reports*.

[3] Minutes of the Phase I Working Group Meeting, NCI, Bethesda, MD, July 1983.



CX-1$_{SC}$
Colon xenografts

colon	+	-
+	0	0
-	0	8

Colon 38

colon	+	-
+	0	0
-	5	3

Anguidine, Pyrazofurin, Maytansine, Chlorozotocin, Deazauridine, Rubidazone, PALA, Amsacrine

B_{16}
melanoma

melanoma	+	-
+	0	0
-	6	2

Anguidine, Pyrazofurin, Maytansine, Chlorozotocin, Rubidazone, PALA, Amsacrine, DON

$^{L}1210$
leukemia

leukemia	+	-
+	4	1
-	3	1

Thymidine, Aclacinomycin, Anguidine, Chlorozotocin, Deazauridine, Rubidazone, Indicine N-Oxide, Amsacrine, Pyrazofurin

LX-1$_{SC}$
lung xenografts

lung	+	-
+	0	0
-	2	6

3^{LL}
Lewis lung carcinoma

lung	+	-
+	0	0
-	2	6

Anguidine, Pyrazofurin, Maytansine, Chlorozotocin, PALA, Amsacrine, DON

MX-1$_{SC}$
breast xenografts

breast	+	-
+	0	1
-	1	6

CD8F$_1$
mammary carcinoma

breast	+	-
+	1	0
-	5	3

Anguidine, Pyrazofurin, Maytansine, Bruceantin, PALA, Amsacrine, Mitoxantrone, Chlorozotocin

FIGURE 1.—Correlation of activity of 10 antitumor agents in murine model tumor systems with activity in human cancer. Activity in murine tumors was judged according to NCI Decision Network 2 criteria (Goldin A, et al. Eur J Cancer 17:129-142, 1981). Activity in human tumors is defined as a 20% response rate in at least 1 clinical trial with ⩾ 14 evaluable patients.

NCI. Results of an interim analysis of phase II results are available for 13 drugs, which were studied in 180 protocols (table 2). Although these data represent only a fraction of the large NCI experience, certain trends are apparent. First, significant activity of ⩾ 20% was seen only in the lymphomas, leukemia, and breast carcinoma (50%, 29%, and 14% of the studies, respectively); most of the results were in the 20%-30% range. No drug showed > 20% activity in colon carcinoma and melanoma, and only 6% of the lung cancer trials showed positive activity. Even with only those drugs which have shown activity in at least one tumor type, the overall response rate in colon and lung cancers and melanomas is still consistently < 10%.

It is well known that colon cancer and melanoma are highly resistant diseases, and that these diseases which are consistently refractory to all therapies are of no value in screening. Although more data are needed, the results to date suggest that inclusion of colon cancer and melanoma in the panel may not be useful for screening. However, it would seem reasonable to continue testing new agents for activity in those common and refractory neoplasms until truly reliable screens for activity have been defined. Such screens may emerge from further analysis of data for clinical trials or from advances in the use of in vitro or in vivo laboratory methods.

Second, even in intrinsically sensitive diseases like

TABLE 2.—Outcome of phase II studies in human cancer

Disease	Total No. of studies	Response rate 0%	< 20%	⩾ 20%
Breast cancer	30	13 (43%)	13 (43%)	4 (14%)
Colon cancer	38	25 (66%)	13 (34%)	0
Leukemia	21	6 (29%)	9 (48%)	6 (29%)
Lung cancer	47	27 (57%)	17 (36%)	3 (6%)
Lymphoma	18	4 (22%)	5 (28%)	9 (50%)
Melanoma	26	16 (62%)	10 (38%)	0
Total	180	91 (51%)	67 (37%)	22 (12%)

breast or small cell lung cancer, the number of drugs showing activity turned out to be very small. As seen in table 3, of the 11 drugs considered in breast cancer, only bisantrene showed an overall response rate of > 20%. As has been true throughout the history of medical oncology (27), the estimates of activity vary widely from trial to trial. For example, with mitoxantrone, response rates ranged from 5% to 28%. This observation suggests the well-known importance of factors other than drug dose and schedule as major influences on estimates of response rate. Again, for mitoxantrone, the deleterious effect of prior therapy on response seems to be fairly clear (table 4).

In fact, the success of the human tumor panel in predicting (or ruling out) general patterns of efficacy for other human cancers will depend to a large extent on what kinds of patients with "panel cancers" are chosen for entry in the study. A negative trial of a new drug in 20 patients with breast cancer who have failed multiple prior regimens tells us nothing about either the potential of this drug in a more favorable breast cancer population or drug activity in other tumors. Moreover, data from earlier eras of cancer chemotherapy cannot be used reliably to decide which tumors may be usefully included in a panel without extensive consideration of how shifting patterns of practice may have altered important patient characteristics.

The phase II effort also needs certain administrative refinements. Table 5 shows a breakdown by disease of patient accrual patterns for negative phase II studies, ie, trials yielding a < 10% response rate. Even allowing for the histologic heterogeneity of certain primary sites such as lung, the extent of overaccrual in some of these categories suggests the need for much earlier review of the data by investigators and a tighter system of control by the statistical offices of cooperative groups. Indeed, several groups have already implemented procedures which should minimize the chances that patients will be entered in treatments already shown to be inactive.

TABLE 3.—Activity of 11 NCI drugs in patients with breast cancer (1975–1980)

Drug	No. of responding patients/ total evaluable	Response rate (%)
Aclarubicin	1/48	2
Amsacrine	12/173	7
Anguidine	1/37	3
Acivicin	0/15	0
Bisantrene	13/50	26
Bruceantin	0/15	0
Diaziquone	2/63	3
Mitoguazone	4/104	4
Mitoxantrone	16/182	9
PCNU	0/45	0
Piperazinedione	3/47	6

TABLE 4.—Responses to mitoxantrone in carcinoma of the breast trials according to previous treatment

Response rate (%)	No. of previous regimens	Institution*
3	3	SWOG
10	3	SECSG
5	2.6	ECOG
22	3	M. D. Anderson Hospital and Tumor Institute
21	1	Ohio State University
19	1	EORTC
28	0	The Royal Marsden Hospital

*SWOG = Southwest Oncology Group; SECSG = Southeastern Cancer Study Group; ECOG = Eastern Cooperative Oncology Group; and EORTC = European Organization for Research on Treatment of Cancer.

Phase III

Once the activity of a compound is established in one or more diseases, subsequent development of the drug proceeds along two separate lines. One of these lines is to establish the role of the drug in the disease for which activity was demonstrated. The endpoints of such studies, which are designed to compare the drug alone or in combination against standard treatment in a randomized fashion, are not only relative activity (eg, response rate), but also response duration, survival, and toxicity; the ultimate goal is to define the specific contribution of the drug in the treatment of a particular cancer. The data from such trials may be used by pharmaceutic firms seeking New Drug Application (NDA) approval from the Food and Drug Administration (FDA) for marketing purposes.

In this connection, the intense interest in chemical analogs of existing active agents poses special challenges to clinical drug development. Of 31 drugs developed by NCI since 1975, eight have been analogs of commercially available or experimental drugs. Until very recently, the development of analogs proceeded along essentially the same lines as that of novel structures. Formal prospective comparisons of analog versus parent were rarely carried out (28). As a result, little direct comparative data exist on the relative merits of the various bifunctional alkylating agents, nitrosoureas, anthracyclines, or epipodophyllotoxins.

Surely, if parent and analog have borderline activity in a certain cancer, such direct comparisons are probably not worth undertaking. Moreover, when such comparisons are worth doing, the trials need to be quite

TABLE 5.—Patient accrual onto negative phase II studies using 13 compounds[*]

	No. of studies				
Disease	Total	< 25 patients	25–50 patients	≥ 50 patients	Median No. of patients
Breast cancer	10	1 (10%)	7 (70%)	2 (20%)	32
Colon cancer	9	2 (22%)	6 (66%)	1 (11%)	31
Leukemia	5	2 (40%)	2 (40%)	1 (20%)	35
Lung cancer (non-small cell)	15	2 (13%)	6 (40%)	7 (47%)	45
Lymphoma	1	0	1	0	(31)
Melanoma	8	2 (25%)	5 (62%)	1 (12%)	35

[*] Aclarubicin, bisantrene, amsacrine, anguidine, acivicin, diaziquone, bruceantin, mitoxantrone, DON, mitoguazone, PCNU, piperazinedione, and zinostatin.

large, because multiple endpoints are involved. Also, since current analog programs, especially for platinum compounds and anthracyclines, are undertaken to obviate major organ toxic effects, design of comparative trials must permit the simultaneous assessment of relative toxicity and efficacy. This task may be particularly difficult if one is dealing with equivalent degrees of efficacy in the presence of decreased toxicity. In such a case, the existence of reduced toxicity is medically important only if no material loss of efficacy has occurred. Clearly, accrual should be large enough that such a conclusion can be drawn with reasonable power.

Another significant problem with orderly analog development is the current embarrassment of riches: analogs are much easier to synthesize than to test comprehensively in the clinic. Accordingly, we now have a situation in which the number of worthy platinum compounds, anthracyclines, and antifols may actually exceed our capacity for rigorous and comprehensive comparative trials.

An example of the NCI's current strategy is the development program for two analogs of cisplatin, CHIP and carboplatin. The focus of their development is in cancers for which the parent compound is active, but not curative, such as carcinomas of the head and neck, uterine cervix, and bladder. In these disorders, randomized trials to compare the two analogs are initiated immediately upon completion of phase I; the superior analog is then to be compared to the parent compound. The trial design includes provisions for early stopping based on absence of efficacy. Nonrandomized phase II trials are implemented in diseases which are less sensitive to cisplatin, such as non-small cell lung or colorectal cancer. The results of these trials will show whether the spectrum of activity with the analogs is extended significantly compared to the parent.

It is also useful to consider within phase III the multitude of exploratory studies that go on with a new agent after conclusion of phase II. These studies include: incorporation of the new drug into combinations, exploration of alternate routes of administration, and use of the agent in high doses accompanied by "rescue" procedures. Such studies, particularly the combination trials, constitute a large proportion of clinical research in cancer. It would certainly be churlish not to acknowledge the many advances that have come from such studies, including many of the crucial advances in medical oncology (2,3,5). Protection of the kidneys from platinum-induced damage (13) and current efforts with intracavitary chemotherapy (29) and intra-arterial chemotherapy (30) are other examples of "post-phase II" drug development that either have been proved valuable already or appear particularly promising.

However, it is equally clear that much of the work in phase III, particularly studies with drug combinations, has been disappointing in concept, design, or execution. Perhaps influenced by the success in certain leukemias and lymphomas, investigators have all too often attempted to hit the home run against tumors which have repeatedly proven refractory to easy solutions. The vast clinical literature in most solid tumors bears witness to the inefficacy of this approach, at least with combinations of drugs having individually low complete response rates. At the end of such uncontrolled "pilot" studies, one is almost always left with efficacy and toxicity data having no clear point of reference. The true pilot study does exist, of course, and is an invaluable tool for testing the feasibility of an approach. Beyond the question of feasibility, however, a clinical trial is *always* comparative in intent, simply because there is always another way to treat the patient. Therefore, the issue of a suitable control group will never go away, no matter how difficult or inconvenient it is to deal with. Whether the control group should always (ie, wherever possible) be selected by a random process or whether under certain circumstances nonrandomized controls can serve nearly as well is, in a sense, less important than the somewhat more basic notion that the use of an explicit control group must be much more pervasive and sophisticated than is now common practice.

Coordination of the Process

For many years, the NCI has been the single largest contributor to all aspects of anticancer drug development. Because of its multifaceted involvement (from organic synthesis and natural product screening through phase III clinical trials) and the sheer volume of its support for the total effort toward drug development, the NCI has functioned, in effect, as the national coordinator of drug development in the US. In the past, the pharmaceutic industry has been less heavily involved in the development of antineoplastics than in other areas. Recently, however, with the growth of clinical oncology as a specialty and the far wider application of chemotherapy in the treatment of cancer, the industry has dramatically increased its interest in cancer.

At the outset, one must recognize that the goals of the NCI and pharmaceutic industry share an important common feature: to define the activity of a new agent and make it available to patients as expeditiously as possible. It is only in this way that the population at large will benefit maximally from the fruits of research. However, one must also recognize the considerable differences in emphasis that the NCI and pharmaceutic industry often bring to this task. In its capacity as a financially disinterested supporter of basic and clinical research, the NCI wishes to see all reasonable steps taken to ensure that the therapeutic potential of a new agent is explored fully. By contrast, a pharmaceutic firm may be somewhat less inclined to support exploratory trials that have no direct role in securing an NDA for a new drug, and will strive to get a drug to market by the most direct and least costly route possible. Once a drug has been approved by the FDA, the company can deal with the question of whether to expand the indications in the postmarketing period or, as has been more often the case with antineoplastic agents, whether to let the evolution of subsequent results work its own effects on the market.

Obviously, the multiplicity of drug sponsors creates both opportunities and problems. The opportunities are clear enough: at a time when federal funds for research are increasingly difficult to obtain, support from private sources is sorely needed. In addition, many pharmaceutic firms now have, in both basic and clinical areas, excellent professional staff with the competence to formulate and supervise comprehensive drug development programs in cancer.

The problems deal chiefly with the optimal utilization of scarce resources. In recent years, the pharmaceutic industry has relied heavily on the same pool of clinical investigators who are funded by the NCI via its peer-review mechanism, which obviously provides private sponsors with an important assurance of competence. On the other hand, industry-funded trials may possibly lead to difficulties in the peer-review process itself, for both scientific and financial reasons. At least some trials which are "NDA directed" may not appear very exciting or innovative when examined for the renewal of an institutional or cooperative group grant. The availability of financial support from industry presents investigators with the powerful incentives to perform trials which may be inherently uninteresting and unlikely to move the field forward. The existence of multiple sources of private support in addition to federal funding creates the necessity for very careful accounting of how funds are spent, to avoid the comingling of private and federal funds.

The NCI has attempted to evolve constructive relationships with the many pharmaceutic firms involved in clinical cancer research. Specifically, this involves much advanced joint planning to assure the orderly development of new agents of mutual interest in a manner which satisfies the needs of pharmaceutic industry for filing of an NDA with the FDA, while simultaneously preserving the autonomy of the clinical cooperative groups. As the chief source of funding for the cooperative groups, the NCI's primary concern is that the availability of support from the private sector neither serve to decrease the scientific quality of group studies nor alter the direction of a group for other than scientific reasons.

CONCLUSION

Up to the present time, the process of antineoplastic drug development in the clinic has been largely an empiric effort. Dose escalation in phase I, definition of an activity spectrum in phase II, and the construction of specific combinations of agents have commonly proceeded without seminal input from the laboratory. This is not to say that experimental results have played no role in the design of productive approaches to cancer treatment; the work of Skipper and Schabel, for example, has had a profound impact on how clinicians think about combination chemotherapy or about the treatment of minimum residual disease. Nevertheless, when a clinical investigator goes about putting together a combination regimen, he may be guided by evidence of synergism in laboratory models, but the final choices of agents, doses, schedules, and sequencing are usually made on the basis of considerations other than hard science. Viewed in this light, the distance we have come in the last 30 years is remarkable; probably no other area of medicine has achieved so much on the basis of empiricism.

In the next few years, we can look forward to a closer integration of the laboratory and the clinic. From a more detailed appreciation of the relation of pharmacokinetics to toxicity in experimental models, it may be possible to devise much more efficient phase I studies, so that fewer patients are treated at biologically ineffective doses. The development of a more complete clinical data base on patterns of response across many histologic types, together with the further evaluation of in vitro techniques for assaying drug sensitivities, may permit a more efficient strategy for identifying the activity of agents in various disease categories. And, as the increasing sophistication of biochemical pharmacology clarifies the mechanisms of action of, as well as the interactions between, antineoplastic agents, one can anticipate the more rational use of drugs, both alone and in combination.

Meanwhile, as we slowly approach the era of total rationality in drug development, we can design a drug development program that is consistent with the current state of our knowledge, however incomplete it may be. Clinical pharmacology studies should always accompany phase I trials, since knowledge of the disposition of parent compound and metabolites may help to determine optimal schedules and dose modification procedures for subsequent study. Patients with extensive prior chemotherapy should not be entered in phase II trials until the activity of a new agent is established for the tumor type in question; what constitutes "extensive" prior treatment must be decided upon for each disease. We plan to emphasize the testing of new agents in previously untreated patients whenever it is ethical to do so, and it is certainly ethical to do so far more frequently than is currently common practice. The NCI will continue to encourage the development of drug combinations which are solidly grounded in a scientific or clinical rationale and for which the proposed clinical trial has a methodology adequate to the objectives of the study.

REFERENCES

1. GOODMAN LS, WINTROBE MM, DAMESHEK W, ET AL. Nitrogen mustard therapy: use of methylbis (β-chlorethyl) amino hydrochloride for Hodgkin's disease, lymphosarcoma, and certain allied and miscellaneous disorders. JAMA 132:126-132, 1946.
2. DEVITA VT, SIMON RM, HUBBARD SM, ET AL. Curability of advanced Hodgkin's disease with chemotherapy: long term follow-up of MOPP treated patients at the NCI. Ann Intern Med 92:587-595, 1980.
3. EINHORN L, and DONOHUE JP. *Cis*-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87:293-298, 1977.
4. FISHER RI, DEVITA VT, HUBBARD SM, ET AL. Diffuse agressive lymphomas: increased survival after alternating flexible sequence of Pro MACE and MOPP chemotherapy. Ann Intern Med 98:304-309, 1983.
5. MAUER AM. Therapy of acute lymphoblastic leukemia in childhood. Blood 56:1-10, 1980.
6. CANELLOS GP, POCOCK SJ, TAYLOR SG, III, ET AL. Combination chemotherapy for metastatic breast carcinoma. Prospective comparison of multiple drug therapy with L-phenylalanine mustard. Cancer 38:1882-1886, 1976.
7. MACDONALD JS, SCHEIN PS, WOOLLEY PV, ET AL. 5-Fluorouracil, mitomycin-C, and adriamycin (FAM). A new combination chemotherapy program for advanced gastric carcinoma. Ann Intern Med 93:533-538, 1980.
8. BONADONNA G, and VALGUSSA P. Chemotherapy of breast cancer: current views and results. Int J Radiat Oncol Biol Phys 9:279-297, 1983.
9. ROSEN G, CAPARROS B, and HUVOS AG. Preoperative chemotherapy for osteogenic sarcoma. Cancer 49:1221-1230, 1982.
10. GLAUBIGER DL, VON HOFF DD, HOLCENBERG JS, ET AL. The relative tolerance of children and adults to anticancer drugs. Front Radiat Ther Oncol 16:42-49, 1982.
11. SLADEK NE, PRIEST J, DOEDEN D, ET AL. Plasma half-life and urinary excretion of cyclophosphamide in children. Cancer Treat Rep 64:1061-1066, 1980.
12. D'INCALCI R, FARINA PL, SESSA C, ET AL. Pharmacokinetics of VP 16-213 given by different administration methods. Cancer Chemother Pharmacol 7:141-145, 1982.
13. CORDEN BJ, HIEL JB, COLLINS J, ET AL. High dose cisplatinum (HDP) in hypertonic saline: absence of nephrotoxicity and pharmacokinetics of 40 mg/m^2 d × 5 schedule of administration. Proc ASCO 2:34, 1983.
14. JONES RB, NORTON L, BHARDWAJ S, ET AL. Single agent adriamycin for metastatic breast cancer: a steep dose-response relationship. Proc ASCO 2:107, 1983.
15. KNIGHT WA, III, PAGE CP, KUHN JC, ET AL. High dose melphalan (L-PAM) with autologous bone marrow (ABM) infusion for Duke's D colo-rectal carcinoma. Proc ASCO 460:118, 1983.
16. LAZARUS HM, HERZIG RH, GRAHAM-POLE J, ET AL. Intensive melphalan chemotherapy and cryopreserved autologous bone marrow transplantation for the treatment of refractory cancer. J Clin Oncol 1:359-367, 1983.
17. FREI E, III, and CANELLOS GP. Dose: a critical factor in cancer chemotherapy. Am J Med 69:585-594, 1980.
18. GRALLA RJ, CASPER ES, KELSEN DP, ET AL. Cisplatin and vindesine combination chemotherapy for advanced carcinoma of the lung: a randomized trial investigating two dosage schedules. Ann Intern Med 95:414-420, 1981.
19. AISNER J, ALBERTO P, BITRAN J, ET AL. Role of chemotherapy in small cell lung cancer: a consensus report of the International Association for the Study of Lung Cancer Workshop. Cancer Treat Rep 67:34-43, 1983.
20. PHILLIPS GL, FOY JW, HERZIG GP, ET AL. Phase I-II study: intensive BCNU (1,3-bis[2-chloroethyl]-1-nitrosurea, carmustine, NSC 409 962) and autologous bone marrow transplantation for refractory cancer. Cancer, in press.
21. MYERS WPL, KARNOFSKY D, and BURCHENAL JH. The hepatotoxic action of *N*-methylformamide in man. Cancer 9:949-955, 1956.
22. DEXTER DL, SPREMULLI EN, MATOOK GM, ET AL. Inhibition of the growth of human colon cancer xenografts by polar compounds. Cancer Res 42:5018-5022, 1982.
23. DEXTER DL, SPREMULLI EN, SAVARESE TM, ET AL. Effects of *N*-methylformamide (N-MF) on murine and human cancer cells. Clin Res 30:53, 1982.
24. LEGHA SS, LATREILLE J, MCCREDIE KB, ET AL. Neurologic and cardiac rhythm abnormalities associated with 4'-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA) therapy. Cancer Treat Rep 63:2001-2003, 1979.
25. HARMON WE, COHEN HJ, SCHNEEBERGER EE, ET AL. Chronic renal failure in children treated with methyl CCNU. N Engl J Med 300:1200-1203, 1979.
26. Application of the human tumor stem cloning assay to drug screening. RFP NCI-CM-07327, Bethesda, MD, National Cancer Institute, Apr 1980.
27. TANNOCK I, and MURPHY K. Reflection on medical oncology: an approach for better clinical trials and improved reporting of their results. J Clin Oncol 1:66-70, 1983.
28. CARTER SK. The clinical evaluation of analogues (I) the overall problem. Cancer Chemother Pharmacol 1:69-72, 1978.
29. MARKMAN H, GREEN MR, PFEIFRE CE, ET AL. Combination intracavitary (IC) chemotherapy in patients with stage III-IV ovarian car-

cinoma failing standard treatment regimens. Proc ASCO 2:147, 1983.

30. ENSMINGER W, NIEDER HUBER J, GYVES J, ET AL. Effective control of liver metastasis from colon cancer with an implanted system for hepatic arterial chemotherapy. Proc ASCO 1:94, 1982.

Drug Resistance in Cancer

Gregory A. Curt,* Neil J. Clendeninn, and Bruce A. Chabner[1]

The development of drug resistance by malignant cells is both a common clinical problem and an area of active research interest. This topic has obvious practical importance, since the appearance of a single drug-resistant mutation could lead to treatment failure. The eventual clonal expansion of the resistant cell may be modulated by independent factors such as immune surveillance, growth rate, and metastatic potential. Nonetheless, it is a common clinical experience to observe the outgrowth of highly drug-resistant tumors in patients initially sensitive to therapy, particularly patients with acute leukemia, diffuse histiocytic lymphoma, and small cell carcinoma of the lung. Elucidation of specific resistance mechanisms in vitro and in animal models (table 1) has provided clues as to possible resistance mechanisms in man and has suggested modification in the design of new agents and new dosing schedules. However, at this point the understanding of mechanisms of drug resistance at the clinical level is woefully incomplete and fragmentary.

In many ways, conceptual developments in this field have paralleled studies of drug resistance in bacteria. The most cogent theories regarding drug resistance in chemotherapy have been offered by Goldie and Coldman (1), using principles initially developed in antimicrobial research. They have developed a model for drug resistance in tumor cell populations, in which the likelihood of finding a single cell with resistance to a specific drug is related to both population size and mutation frequency. Resistant cells are not "induced" by the toxic agent, except perhaps in the case of true mutagens such as alkylating agents, but rather occur spontaneously as mutants at a predictable frequency in cell populations. Drug exposure, then, provides a positive selective pressure, allowing the resistant cells to outgrow the sensitive tumor cell population. Taken to its logical conclusion, this model predicts optimal treatment results using drug combinations rather than single agents (as is common in the treatment of infection, eg, tuberculosis) and treating at an early stage in disease when the tumor burden is small.

Indeed, general mechanisms of bacterial resistance to antibiotics—such as increased drug inactivation (penicillin), decreased drug permeability (amino acid analogs), altered target enzyme affinity for drug (sulfonamides), and altered drug receptor (streptomycin)—find their parallel in cancer cells (although, of course, the specific enzymes or target proteins differ), while other mechanisms, such as decreased drug activation, have thus far been described only in tumors.

In this review we will discuss mechanisms of resistance to antitumor agents and potential strategies for their reversal. The known mechanisms of drug resistance in cancer cells include: (*a*) defective transport, (*b*) defective drug metabolism to active species, (*c*) altered intracellular nucleotide pools, (*d*) increased drug inactivation, (*e*) altered DNA repair, (*f*) gene amplification, and (*g*) altered target protein. As can be seen from table 1, resistance to a given drug can be effected by a number of independent mechanisms, while resistance to a specific agent can confer cross-resistance to structurally dissimilar drugs with different mechanisms of action (pleiotropic drug resistance). The pathway by which cells acquire pleiotropic drug resistance appears to be at the level of membrane transport.

Indeed, altered intracellular accumulation of tumoricidal drugs is probably the most important general mechanism of resistance, affecting nearly all classes of antitumor agents, with the possible exception of the highly lipophilic alkylators such as the nitrosoureas. Ironically, drug transport remains poorly understood, having been characterized for most drugs in purely kinetic terms, without identification of responsible membrane components.

Our need to understand the biochemical, pharmacologic, and genetic mechanisms of drug resistance arises from the possibility that novel treatment strategies using currently available agents might circumvent or delay the development of resistance. Further, a more complete understanding of the intricacies of drug resistance might suggest new agents or drug combinations specifically lethal to resistant cells.

---

[1] Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Gregory A. Curt, MD, Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 6N119, Bethesda, MD 20205.

TABLE 1.—Mechanisms of resistance to cytotoxic drugs

General mechanism	Drug	Specific alteration (ref Nos.)
Defective transport	Methotrexate	Decreased carrier-mediated uptake (2-6)
	Melphalan Mechlorethamine	Decreased carrier-mediated uptake (61,62)
	Cytarabine	Decreased membrane nucleoside binding sites (40)
	Doxorubicin	Increased efflux (120,121)
Defective drug metabolism to active species	Cytarabine	Decreased deoxycytidine kinase activity (32-34)
	5-Azacitidine	Decreased uridine-cytidine kinase activity (44,45)
	5-FU	Decreased uridine kinase activity, decreased orotic acid phosphoribosyltransferase, decreased uridine phosphorylase (46-49)
	6-Mercaptopurine 6-Thioguanine	Decreased HGPRT activity (92)
	Methotrexate	Defection polyglutamation (28,29)
	Doxorubicin	Decreased P450 or flavin reductase (115)
Increased drug inactivation	6-Mercaptopurine 6-Thioguanine	Increased membrane alkaline phosphatase (93-96)
	Cytarabine	Increased cytidine deaminase activity (35-37)
	Alkylators	Increased intracellular glutathione (65-71) or metallothionein (75)
	Bleomycin	Increased bleomycin-hydrolase activity (112,113)
	Cisplatin	Increased intracellular metallothionein (88)
	Doxorubicin	Increased intracellular degradation (111)
		Increased intracellular glutathione (116,117)
Altered DNA repair	Alkylators Cisplatin Doxorubicin	Increased efficiency of excision of damaged bases and/or ligation of excised segment (78-82,87)
Gene amplification	Cadmium	Increase in metallothein gene copy number (74)
	PALA	Increase in aspartate transcarbamylase gene copy number (124)
	Methotrexate	Increase in DHFR gene copy number (16-26)
	Doxorubicin	Association of unstable resistance with double-minute chromosomes (? gene product) (119)
	5-FU	?Increase in thymidylate synthetase gene copy number (52-54)
	Pentostatin	? Increase in adenosine deaminase gene copy number (125)
Altered targets	Methotrexate	Altered DHFR (10-15)
	Vincristine	Altered tubulin (100-104)
	5-FU	Altered thymidylate synthetase (51)
	Hydroxyurea	Altered ribonucleotide reductase (126)
	Steroids	Altered steroid receptor (97)
		Altered steroid receptor complex binding to DNA (98)
	Doxorubicin	Decreased affinity of membrane lipids for drug (110)
Altered nucleotide pools	Cytarabine	Increased intracellular CTP and dCTP pools (38)
Salvage pathways	Methotrexate	Increased purine salvage (30,31)
	5-FU	Increased thymidine kinase activity (55)
Pleiotropic drug resistance	Doxorubicin Vinca alkaloids Dactinomycin	Defective drug accumulation due to increased energy-dependent efflux (130-132) ?Specific membrane glycoprotein markers (126-132,137-139)

Methotrexate (MTX)

MTX has become a model compound in the study of mechanisms of tumor cell resistance. An analog of folic acid, MTX is transported intracellularly by the membrane carrier responsible for uptake of physiologic folates and inhibits its target enzyme, dihydrofolate reductase (DHFR), interfering with thymidine and de novo purine synthesis (fig 1). Resistance to MTX has been attributed to altered transport, altered target protein, gene amplification, defective metabolism to polyglutamate species, and availability of salvage pathways.

In 1965 Kessel and co-workers (2) demonstrated that MTX uptake is a critical determinant of response in murine leukemia. Since then, MTX-resistant tumor cell lines with defective drug transport have been established by serial selection, both in vitro (3–5) and in tumor-bearing animals (6). Of interest is the fact that the transport defect in MTX-resistant cells can be overcome in the presence of high ($> 10\ \mu$M) extracellular drug concentrations (7) which promote uptake of drug by diffusion. These observations constitute a rationale for high-dose MTX in clinical trials. A second approach has been the design of lipid-soluble antifolates, such as trimetrexate (8) and esters of MTX (9), which can also enter cells by diffusion to overcome transport resistance.

Altered target protein has been extensively studied as a mechanism of MTX resistance. MTX-resistant cells in which DHFR has a decreased affinity for drug have been isolated in both animal and human cells in vitro (10–15). The altered DHFR differs only slightly from wild-type enzyme in molecular weight (15) but has affinities for drug which are 2.5- to 270-fold decreased, a change likely mediated by amino acid substitutions at the enzymatic active site.

A unique mechanism of mutation to drug resistance is amplification of the gene coding for target enzyme, resulting in increased mRNA copies and cellular levels of that protein. Gene amplification is believed to occur independent of drug exposure but only confers a survival advantage in the presence of a drug which interacts with the amplified gene product. It is possible, however, that drugs such as MTX and other inhibitors of DNA synthesis may cause random repetition of DNA sequences during recovery from drug effects.

MTX-resistant tumor cells with amplified DHFR gene copies may be characterized by specific karyotypic changes. The amplified genes may reside in tandem on a

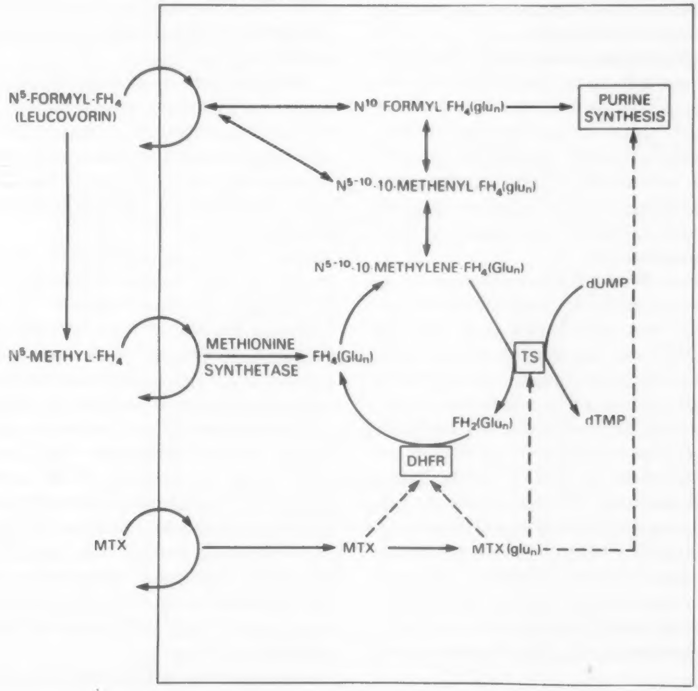


FIGURE 1.—Mechanism of action of MTX. TS = thymidylate synthetase. Dotted lines indicate enzyme inhibition.

single chromosome to produce an expanded "homogeneously staining region" to which copies of the DHFR gene can be localized by hybridization with nucleic acid complementary to the DHFR gene (16-18). This form of resistance is genetically stable, since the amplified genes are integrated with chromosomal DNA and passed to both daughter cells during mitosis. Alternatively, the amplified genes may be located in extrachromosomal DNA fragments called double-minute chromosomes. These paired, spherical pieces of DNA lack centromeres and segregate randomly during division. Cells containing double minutes persist in culture in the presence of selective drug pressure, but in the absence of drug tend to be lost. Thus, drug resistance associated with gene amplification in double-minute chromosomes is unstable in the absence of the selecting agent (19-22).

Double-minute chromosomes coding for DHFR are akin to the extrachromosomal resistance transfer factors which carry genes for antibiotic resistance in bacteria. Like resistance transfer factors, double-minute chromosomes encapsulated in plasmids can be used to transform cells. O'Hare and co-workers (23) have used plasmids containing prokaryotic DHFR to transfer MTX resistance to mouse fibroblasts, while Cline et al (24) have extended the technique to bone marrow cells in intact animals. Thus, currently available technology may allow the selective genetic manipulation of host cells to improve the therapeutic index of tumoricidal agents.

Indeed, gene amplification as a mechanism of mutation to drug resistance is not only an in vitro laboratory phenomenon, but occurs in vivo as well. A small cell lung cancer line established from a patient in relapse following high-dose MTX demonstrated unstable drug resistance in association with double-minute chromosomes (25), while the peripheral blast cells of a patient with leukemia receiving intrathecal MTX were found to have amplified gene copy numbers (26).

As more is understood about a drug's mechanism of action, further insights into resistance mechanisms are possible. It has recently been appreciated that, like the physiologic folates, MTX can be metabolized to γ-polyglutamate derivatives which accumulate intracellularly in large quantities, are preferentially retained in the absence of extracellular drug, and are capable of causing delayed cytotoxicity (27). Cowan and Jolivet (28) have isolated an MTX-resistant clone of ZR-75B human breast cancer through serial selection. The only significant biochemical difference between the parent line and the resistant subline was the inability of the latter to metabolize MTX to its polyglutamate forms. Further, studies of human small cell carcinoma lines isolated from MTX-treated patients have indicated that defective polyglutamation may be an important mechanism of resistance to treatment (29).

Since MTX inhibits both thymidine and de novo purine synthesis, the availability of salvage pathways may be an important determinant of drug resistance. In fact, in vitro evidence suggests more efficient thymidine and purine salvage pathways in normal host tissues, such as bone marrow, compared to tumor cells, and this may be a critical modulator of the therapeutic index of the drug (30). In some tumors, MTX has a predominant antipurine effect. Browman and Csullog (31) selected murine leukemia cells with impaired purine salvage and demonstrated increased sensitivity to MTX in vivo. Cells with impaired purine salvage are more MTX-sensitive because of greater dependence on de novo purine synthesis and inability to overcome antipurine effects by utilizing exogenous purines.

Cytarabine (Ara-C)

The active form of ara-C is the triphosphate metabolite (ara-CTP) which directly inhibits DNA synthesis and can be incorporated into DNA, causing lethal, though poorly defined, effects. The initial metabolism to a monophosphate (ara-CMP) is mediated by the enzyme deoxycytidine kinase (32), and this is believed to be the rate-limiting step in drug activation. In addition, drug can be inactivated by two catabolic enzymes: cytidine deaminase, which degrades ara-C to inactive ara-U; and dCMP deaminase, which catabolizes ara-CMP to inactive ara-UMP. Thus, formation of ara-CTP is modulated by the relative activities of both activating and degradative enzymes.

Decreased activity of deoxycytidine kinase has been reported in numerous ara-C-resistant murine tumors as well as myeloblasts of patients refractory to ara-C (33). As might be expected, this type of drug resistance can be overcome by encapsulation of ara-CTP in liposome carriers, which allows direct intracellular entry of active drug (34).

The activity of degradative enzymes may also be crucial to ara-C resistance. Stewart and Burke (35) reported high cytidine deaminase levels in patients with leukemia and de novo or acquired resistance to ara-C. However, subsequent work has emphasized the heterogeneity of enzyme activities in patient samples, and this single mechanism of resistance is disputed (36,37).

Altered intracellular nucleotide pools may also be a factor in ara-C resistance. High levels of intracellular dCTP (due to decreased dCMP deaminase and/or increased CTP synthetase) can result in ara-C resistance by increasing feedback inhibition of deoxycytidine kinase and competing directly with ara-CTP for incorporation into DNA. Expanded intracellular pools of dCTP have been reported in ara-C-resistant murine cells (38), but the importance of this mechanism in clinical drug resistance remains uncertain.

In studies with human tumor, the intracellular half-life of ara-CTP appears to be critical to drug responsiveness. Rustum and Preisler (39) discovered a positive correlation between ara-CTP retention by leukemic cells in vitro and the duration of complete remission in patients with acute myelogenous leukemia. The specific alteration responsible for the shortened half-life of drug in resistant leukemia was not established, however, and it remains unclear whether formation or retention of ara-CTP is more important.

Impaired drug uptake may also be a determinant of ara-C resistance. Wiley and co-workers (40) have characterized the nucleoside and ara-C transport system in acute leukemia cells and have determined initial ara-C uptake rates to be two to six times greater in leukemia cells compared to mature lymphocytes and granulocytes. Drug uptake rates in individual patient-derived lines correlated well with the relative number of nucleoside transport sites, as determined by binding of a radiolabeled nucleoside analog, ^{3}H-nitrobenzylthioinosine. Moreover, the likelihood of clinical response to ara-C correlated well with the efficacy of drug transport. Of 13 evaluable patients, five failed to achieve a complete clinical remission, and three of these five demonstrated the lowest rates of drug influx. Prospective trials will be necessary to correlate ara-C transport with clinical response and remission duration.

5-Azacitidine

Like ara-C, 5-azacitidine requires phosphorylation for cytotoxic effect. 5-Azacitidine monophosphate inhibits the enzyme orotidylate decarboxylase, resulting in decreased de novo pyrimidine synthesis (41). The diphosphate of 5-azacitidine is a substrate for both ribonucleotide reductase and DNA polymerase, allowing drug incorporation into both RNA and DNA (42). Conversion of 5-azacitidine to its monophosphate is catalyzed by uridine-cytidine kinase, an enzyme found in tumor cells (43). Because levels of this enzyme are low and because it has a much lower affinity for 5-azacitidine than for the physiologic nucleoside, this initial conversion is the rate-limiting step in drug activation. Tumor cells selected for resistance to 5-azacitidine demonstrate a deletion of this rate-limiting enzyme (44,45).

5-Fluorouracil

Two mechanisms exist for 5-FU toxicity: inhibition of thymidylate synthetase (TS) and incorporation into RNA (fig 2). To be incorporated into RNA, 5-FU must first be converted to FUMP either directly by orotic acid phosphoribosyltransferase or through conversion to 5-FUR by uridine phosphorylase and phosphorylation of 5-FUR by uridine kinase. The pathway through orotic acid phosphoribosyltransferase appears to predominate in normal tissue, while tumors may activate the drug by any one, or combination, of the several pathways described above. To inhibit TS, 5-FU must first be converted to 5-FUdR by thymidine phosphorylase and then phosphorylated to 5-FdUMP by thymidine kinase. Alternatively, FdUMP may

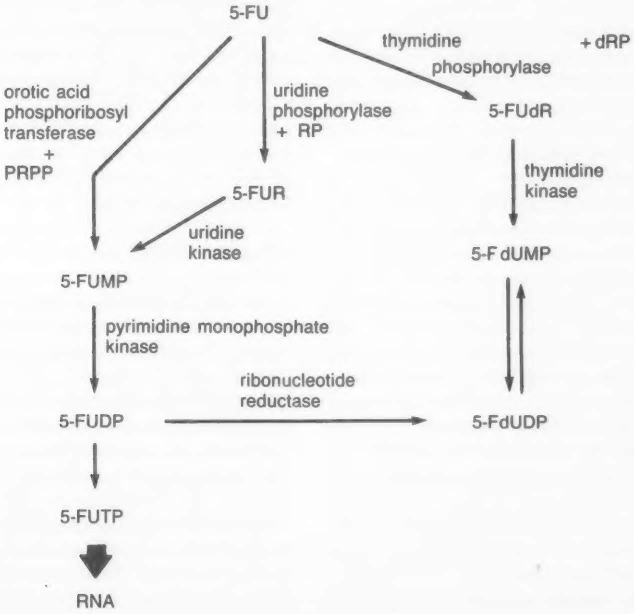


FIGURE 2.—Activation pathways for 5-FU.

be formed by reduction of fluorouracil ribonucleotides (FUDP) by ribonucleotide reductase. Formation of the 5-FdUMP-TS complex requires the presence of 5,10-methylenetetrahydrofolate for covalent binding.

Deletion of activating enzymes, including uridine kinase (46), orotic acid phosphoribosyltransferase (47,48), and uridine phosphorylase (49), has been described in 5-FU-resistant cells as well as in decreased levels of intracellular folates required for 5-FdUMP-TS complex formation (50).

Alterations in TS itself can also confer 5-FU resistance. Resistant cells have been described in which the binding affinity of TS for 5-FdUMP is decreased (altered target) (51). In addition, elevated levels of TS have been reported in 5-FU-resistant fibroblasts (52), neuroblastoma (53), and hepatoma (54) cells. An answer as to whether elevated target protein levels in these cells represent a product of gene amplification or some other mechanism (increased mRNA stability or efficiency of transcription or increased enzyme half-life) awaits further study. When TS is inhibited, the activity of thymidine kinase, which salvages thymidine to bypass enzymatic blockade, is another determinant of resistance (55). The complexity of 5-FU metabolism and the difficulty in analyzing each of the various factors in human material have prevented the elucidation of resistance mechanisms at a clinical level. The reader is referred to recent reviews of this field for more detailed information (56).

Alkylating Agents

The classical alkylating agents function by covalent binding of alkyl moieties to cellular constituents. Several mechanisms of tumor resistance to these agents have been proposed.

Melphalan and mechlorethamine enter cells by carrier-mediated, energy-dependent transport (57,58), with melphalan utilizing the leucine transport system (59) and mechlorethamine sharing the carrier responsible for choline transport (60). Mutant cell lines with defective drug uptake and resultant acquired resistance have been described for both agents (61,62). However, since the transport carrier is different for each drug, tumor cells with transport-dependent resistance to one alkylator may retain sensitivity to others (63), an observation consistent with clinical experience where responses to second alkylators after disease progression on a first are commonly observed. Transport resistance has not been described for lipophilic alkylators such as carmustine and lomustine, which appear to enter cells via passive diffusion (64).

Acquired resistance to alkylators may also be due to increased intracellular levels of protein-free sulfhydryl compounds capable of inactivating the drug (65-67). Specifically, glutathione has been found to be an important modulator of response to cyclophosphamide (68) and nitrosoureas (69).

Recently, Suzukake and co-workers (70,71) have shown that melphalan-resistant murine leukemia cells have increased intracellular levels of glutathione and glutathione disulfide, which are critical for dechlorination of melphalan to a nontoxic metabolite, 4-[bis(2-hydroxyethyl)-amino]-L-phenylalanine. Although other investigators had previously demonstrated that increased metabolism of alkylators might be an important modulator of resistance (72,73), an exciting observation was that reduction of intracellular glutathione and glutathione disulfide in resistant cells by nutritional restriction of L-cysteine could result in a restoration of alkylator sensitivity (70). Other detoxification mechanisms such as an increase in intracellular metallothionein [a sulfhydryl-rich protein which can sequester heavy metals (74) and alkylators] have been described in chlorambucil-resistant cells (75).

Since alkylating agents can cross-link DNA and alkylate nucleic acids and nucleotides, the effectiveness of DNA repair following alkylator damage may also determine treatment response. However, it is important to remember that DNA repair is a complex process which requires excision of alkylated bases, resynthesis of the excised segment by a specific DNA polymerase, and ligation of the new segment. In early work in this area, enhanced excision of alkylated nucleotides from DNA appeared to be responsible for bacterial resistance to alkylating agents (76,77), and this resistance mechanism was subsequently demonstrated in mammalian cells as well (78). Similarly, in a cultured melanoma cell line, alkylator resistance appears to be mediated by enhanced excision (79), while in human glioblastoma cells, increased activity of a specific excision enzyme, guanine-O^6-alkyl-transferase, is correlated with in vitro resistance to nitrosoureas (80). In the few other studies of repair of drug-induced DNA alkylation, DNA cross-links induced by busulfan disappear more rapidly in resistant cells (81), while in murine sarcoma, resistance may be related to efficiency of ligation (82).

Cisplatin (CDDP)

As with the alkylators, one of the major factors mediating the cytotoxicity of CDDP is probably the formation of cross-links between opposing strands of DNA (interstrand), within a single strand of DNA (intrastrand), or between DNA and protein. Although bacterial resistance to CDDP appears to be due to increased efficiency of excision of the damaged sequence (83), the details of tumor cell resistance are less certain. In studies of CDDP responsiveness, Zwelling et al (84), Ducore et al (85), and Erikson et al (86) have used alkaline elution techniques to demonstrate a direct relationship between DNA cross-linking and tumor cell survival. However, the precise mechanisms whereby cells become platinum-resistant are obscure. A CDDP-resistant L1210 line is known to form drug-induced cross-links at a reduced rate, suggesting either slower drug uptake, impaired activation, or rapid repair of DNA damage (87). As with the alkylators, high levels of intracellular metallothionein are found in CDDP-resistant cells (88). This small (molecular weight = 6000), cysteine-rich protein is a logical mediator of CDDP resistance, since it is essential to the detoxification of cadmium and other heavy metals (74). Although intracellular platinum appears to bind metallothionein in resistant cells, it is uncertain whether this represents intact drug or other platinum species. Because the cellular mechanisms of resistance to CDDP are so poorly characterized, new platinum analogs in clinical trial have been selected in an attempt to reduce host toxicity rather than to overcome specific mechanisms of resistance (89).

6-Thiopurines

6-Mercaptopurine (6-MP) and 6-thioguanine require conversion to their ribose phosphates for cytotoxic effect (90). This reaction is catalyzed by hypoxanthine-guanine phosphoribosyltransferase (HGPRT), the enzyme responsible for salvage of physiologic purines (91). Resistance to these agents in murine tumors in vitro has been ascribed to decreased HGPRT levels (92). However, in vitro mechanisms of resistance do not necessarily find their in vivo parallel. In the only detailed studies of resistance to 6-MP in human cells, resistance was mediated by increased activity of membrane-associated alkaline phosphatase, which degrades 6-MP nucleotides in inactive 6-thioinosine (93,94). It is noteworthy that the degradation enzymes of resistant cells differ in their physical-chemical and immunologic properties from the alkaline phosphatase of sensitive cells, suggesting the expression of a new gene product (95,96).

Steroids

Steroids have multiple effects on cellular economy and are cytotoxic to some tumor cells. These effects are mediated by the initial interaction of steroid with a specific cytoplasmic receptor. The steroid-receptor complex then undergoes internal modification (possibly phosphorylation) and translocates to the nucleus, where it modulates DNA transcription to affect mRNA translation and protein synthesis.

While the most frequent clinical explanation for steroid resistance is absence of the cytoplasmic receptor (eg, receptor-negative breast cancer), resistance may be mediated by either altered binding affinity of cytoplasmic receptor for steroid (97) or defective binding of the steroid-receptor complex to DNA (98). Other possible mechanisms of steroid resistance include failure of the complex to undergo internal modification and inability to enter the nucleus or bind chromatin. In fact, even when all steps necessary for steroid activity are intact, a defect in final message translation may exist. These so-called "post-receptor" alterations may explain the presence of adequate numbers of normal receptors in steroid-resistant cells (99).

Antitubulin Agents

These agents, including the clinically important vinca alkaloids and their analogs, inhibit cell division by binding to the protein, tubulin, and preventing its polymerization to microtubular structures. Changes in cytoskeletal proteins can confer resistance to the tubulin inhibitors vincristine, vinblastine, demecolcine, and colchicine. These changes include decreased tubulin binding affinity for drug, changes in the physical (electrophoretic) properties for tubulin subunits, and altered microtubule-associated proteins (100–104). In addition, resistance to antitubulin agents may result from decreased drug uptake and be associated with cross-resistance to antitumor antibiotics and dactinomycin. This mechanism will be discussed in detail in the section on pleiotropic drug resistance.

Antitumor Antibiotics

The anthracyclines have several likely mechanisms of action including direct binding to DNA and cell membranes, free radical formation, and chelation of metal ions with resultant damage to DNA and cell constituents (105). Intercalative binding of doxorubicin to DNA has long been considered of primary importance to cytotoxicity. Avidity of DNA binding is directly correlated to cytotoxicity for series of anthracyclines (106), and DNA damage is apparent in drug-treated cells (107). However, other sites of drug action may be important determinants of resistance.

The importance of membrane binding to doxorubicin cytotoxicity was convincingly demonstrated by Tokes and co-workers (108) who covalently coupled doxorubicin to polyglutaraldehyde microspheres. The microsphere-bound drug was equally toxic to three drug-sensitive and two moderately resistant cell lines as free drug, indicating that intracellular anthracycline was not necessary for activity. More recently, these investigators have demonstrated that microsphere-bound doxorubicin also retains activity in highly drug-resistant cultured hepatocytes, suggesting that high local drug concentration at the level of the cell membrane may be an important determinant of cytotoxicity (109). This notion is strengthened by the observation that doxorubicin-sensitive mouse leukemia cells synthesize macromolecular membrane lipids which specifically bind doxorubicin (110). As compared to sensitive cells, a resistant line derived in culture requires ten times more doxorubicin for membrane receptor saturation because of altered affinity for drug. Again, these results support the notion that decreased membrane binding may result in doxorubicin resistance. However, microsphere-bound doxorubicin might also kill resistant tumor cells by protecting drug from intracellular degradation. This mechanism of resistance has been described for both doxorubicin (111) and the antitumor antibiotic bleomycin (112,113).

Once transported intracellularly, doxorubicin may be reduced by cellular enzymes (P450 reductase, flavin reductase) to a free radical semiquinone which then may directly bind DNA and protein or react with oxygen to form cytotoxic oxygen radicals (114). Anthracycline resistance might then result from a decrease in activity of the enzymes responsible for drug reduction (115) or an increase in intracellular glutathione, which may scavenge and detoxify free radical species (116,117).

Altered intracellular drug accumulation may also mediate doxorubicin resistance (118), and this mechanism may be associated with cross-resistance to structurally dissimilar agents. Interestingly, this phenomenon may be the result of gene amplification. Baskin and coworkers (119) have demonstrated that doxorubicin-resistant cells with decreased net drug uptake and multidrug cross-resistance possess double-minute chromosomes. With serial passage in drug-free media, the minute chromosomes and drug permeability barrier were lost in parallel. Although an amplified gene product was not identified, the similarity to gene-amplified MTX resistance remains clear.

However, while defective MTX and alkylating agent transport result from decreased carrier-mediated drug influx, the alteration with doxorubicin appears to be increased active transport of doxorubicin out of tumor cells (120,121). Thus, in the presence of metabolic inhibitors such as dinitrophenol and sodium azide, drug uptake in resistant cells is identical to that of the sensitive parent line, but following restoration of oxidative metabolism with glucose, resistant cells are able to extrude intracellular drug more efficiently.

Still, defective transport cannot explain all cases of anthracycline resistance. In fact, drug-resistant cell lines with increased doxorubicin uptake have recently been described (122,123). The basis of resistance here has yet to be determined. Overall, since the anthracyclines have multiple potential sites of action, it seems unlikely that a single, unified mechanism of resistance will be found.

Miscellaneous Agents

The understanding of cellular resistance to other drugs in clinical use remains fragmentary. The antimetabolite (*N*-(phosphonacetyl)-L-aspartic acid) inhibits aspartate transcarbamylase, and resistance to this drug in cultured cells may occur through target-enzyme gene amplification (124). Similarly, resistance to pentostatin may be mediated by amplification of the gene coding for adenosine deaminase as evidenced by increased mRNA copies for the catabolic enzyme in drug-resistant cells (125). Hydroxyurea resistance appears to result from initial alteration in the binding affinity of ribonucleotide reductase, followed by an amplification of the altered enzyme and greater drug tolerance (126).

Pleiotropic Drug Resistance (PDR)

A novel and particularly interesting phenomenon is the ability of tumor cells to develop PDR. After exposure to a single drug, usually an antitumor antibiotic or plant alkaloid, cells may become cross-resistant to a number of structurally unrelated compounds with different mechanisms of action. This in vitro finding has its clinical parallel: patients with recurrent disease following primary therapy are generally less responsive to salvage programs using presumably non-cross-resistant therapy. The spectrum of drugs to which cross-resistance is expressed includes anthracyclines, vinca alkaloids, and dactinomycin (127,128). While tumor cells exposed to one of these drugs may demonstrate cross-resistance to the others, collateral sensitivity to cyclophosphamide, glucocorticoids, and local anesthetics has been reported (129).

Preliminary studies to determine a single explanation for PDR suggested that resistant cells have impaired ability to accumulate and retain drug, although the precise mechanism of this "altered permeability" remains speculative (130–132). Some workers have suggested that drugs to which resistance is expressed in the PDR phenotype enter cells by passive diffusion but are actually extruded by an energy-dependent efflux pump (133,134). In resistant cells, drug efflux is more efficient, resulting in net lower intracellular drug uptake. However, it remains difficult to understand why an efflux mechanism should exist for compounds which enter cells passively, and how a single membrane carrier could have affinity for such structurally divergent drugs. In none of the studies published to date have the kinetics of the efflux mechanism been characterized.

The membranes of cells with PDR have been the object of intense investigation. In the early 1970s, Kessel and Bosmann (135,136) suggested that acquired drug resistance occurred through selection of cells with altered membranes and altered membrane synthetic apparatus. Specifically, compared to the drug-sensitive parent cell line, drug-resistant cells had higher levels of enzymes responsible for glycoprotein synthesis and lower levels of enzymes responsible for glycoprotein catabolism. This suggested that resistant cells had a "thick" or more impenetrable membrane, analogous to the glycoprotein capsules of antibiotic-resistant bacteria. Moreover, one-dimensional gel electrophoresis of ^{14}C-glucosamine-labeled resistant cells identified newly expressed, high molecular weight glycoproteins, suggesting a phenotypic marker of drug resistance.

More recently, one-dimensional gel electrophoresis of

^{14}C-glucosamine-labeled drug-resistant Chinese hamster cells has been used to describe a high molecular weight (170,000 daltons) "P-glycoprotein" which appears to be a marker for drug resistance. The resistant cells which synthesize the P-glycoprotein were selected in colchicine, have impaired colchicine uptake, and are highly cross-resistant to anthracyclines, vincristine, puromycin, and dactinomycin (129-132). Amplified membrane glycoproteins have been identified in daunorubicin- and vincristine-resistant murine cells with PDR (137,138) as well as vinca alkaloid-resistant human leukemia lymphoblasts (139), although other changes may not be found in intracellular proteins.

Compelling data have been presented (129-132) to suggest that the P-glycoprotein is directly related to the expression of resistance. Individual clones selected for colchicine resistance independently express the same membrane change, and the degree of drug resistance is proportional to the amount of P-glycoprotein expressed. Cells which spontaneously revert to drug sensitivity no longer express the resistance-related membrane alteration. Moreover, calcium-precipitated DNA from resistant cells can be used to transfer PDR and results in expression of the P-glycoprotein in transformed cells (140). However, exposure of resistant cells to pronase (which enzymatically cleaves the resistance-associated protein) or tunicamycin (which inhibits glycoprotein synthesis) does not overcome PDR or increase "drug permeability" (141). These data suggest that the carbohydrate moiety of the P-glycoprotein may not itself directly mediate resistance.

Another interesting characteristic of vinca alkaloid and anthracycline resistance is its apparent reversibility by a number of compounds including calcium channel blockers and calmodulin inhibitors. The calcium channel blocker verapamil (used in the treatment of supraventricular tachyarrhythmias, hypertension, and angina) has been shown to reverse resistance to vincristine in P388 leukemia (142,143) and to doxorubicin in Ehrlich ascites tumor cells (144). Verapamil has been postulated to increase the uptake and/or decrease the efflux of drugs in resistant cells, thereby reversing permeability-dependent resistance (145-147). Similar reversal of resistance has been reported using the calmodulin inhibitors prenylamine, trifluoperazine, and chloripramine (144,148), the calcium channel blockers diltiazem, nicardipine, niludipine, and nimodipine (149), and the antihypertensive reserpine (150).

Whether reversal of PDR by this multitude of agents is related to specific effects on calcium channels and calmodulin or more general effects on calcium-dependent enzyme systems (eg, cyclic AMP, Ca^{++}, Mg^{++}-ATPase) remains to be elucidated. A more thorough understanding of PDR, the mechanisms underlying its reversal, and the potential applicability of this knowledge to clinical cancer treatment are important subjects of ongoing studies.

CONCLUSIONS

At both the experimental and the clinical level, there exist obvious gaps in our understanding of antitumor drug resistance. The processes of drug uptake and extrusion are particularly important and, unfortunately, have yet to be characterized in either a kinetic or structural sense for most of the antibiotics or plant products currently in clinical use. This information would be pertinent to the problem of understanding pleiotropic drug resistance, and the reversal of this resistance by calcium channel blockers. Other forms of resistance, particularly those involving deletion or amplification of enzymes crucial to drug activity, are better understood from a biochemical standpoint, but their relevance to clinical drug resistance has not been firmly established by the necessary prospective patient studies which correlate response with tumor cell biochemistry. In particular, the process of antimetabolite nucleotide degradation—which seems to be an important determinant of response to thiopurines, ara-C, and fluoropyrimidines—requires more careful study.

Finally, additional attention must be directed toward understanding the genetic basis of drug resistance. Why is resistance so easily induced in tumor cells but not in normal proliferative tissues? What factors, such as mutagenic drugs, increase the frequency of drug resistance? Are there specific clinical strategies which can be used to discourage the outgrowth of resistant cells? These questions, amenable to study in experimental systems, have obvious relevance to clinical treatment planning and drug usage.

REFERENCES

1. GOLDIE JH, and COLDMAN AJ. A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 63:1727-1733, 1979.
2. KESSEL D, HALL TC, and ROBERTS D. Uptake as a determinant of methotrexate response in mouse leukemia. Science 150:752-754, 1965.
3. HARRAP KR, HILL BT, FURNESS ME, ET AL. Sites of action of amethopterin: intrinsic and acquired drug resistance. Ann NY Acad Sci 186:312-324, 1971.
4. HILL BT, BAILEY BD, WHITE JC, ET AL. Characteristics of transport of 4-amino antifolates and folate compounds of two lines of L5178Y lymphoblasts, one with impaired transport of methotrexate. Cancer Res 39:2440-2446, 1979.
5. OHNOSHI T, OHNUMA T, TAKEHASHI I, ET AL. Establishment of methotrexate-resistant human acute lymphoblastic leukemia cells in culture and effects of folate antagonists. Cancer Res 42:1655-1660, 1982.
6. SIROTNAK FM, MOCCIO DM, KELLEHER LE, ET AL. Relative frequency and kinetic properties of transport-defective phenotypes among methotrexate-resistant L1210 clonal cell lines derived in vivo. Cancer Res 41:4447-4452, 1981.
7. HILL BT, DEDHAR S, and GOLDIE JH. Evidence that at "high" extracellular methotrexate concentrations the transport barrier is unlikely to be an important mechanism of drug resistance. Biochem Pharmacol 31:263-266, 1982.

8. OHNOSHI T, OHNUMA T, TAKAHASHI I, ET AL. Establishment of methotrexate-resistant human acute lymphoblastic leukemia cells in culture and effects of folate antagonists. Cancer Res 42:1655-1660, 1982.
9. CURT GA, TOBIAS JS, KRAMER RA, ET AL. Inhibition of nucleic acid synthesis by the di-*n*-butyl ester of methotrexate. Biochem Pharmacol 25:1943-1946, 1976.
10. GOLDIE JH, KRYSTAL G, HARTLEY D, ET AL. A methotrexate-insensitive variant of folate reductase present in two lines of methotrexate-resistant L5178Y cells. Eur J Cancer 16:1539-1546, 1980.
11. JACKSON RC, and NIETHAMMER D. Acquired methotrexate resistance in lymphoblasts resulting from altered kinetic properties of dihydrofolate reductase. Eur J Cancer 13:567-575, 1977.
12. HANGGI UJ, and LITTLEFIELD JW. Isolation and characterization of the multiple forms of dihydrofolate reductase from methotrexate-resistant hamster cells. J Biol Chem 249:1390-1397, 1974.
13. FLINTOFF WF, and ESSANI K. Methotrexate-resistant Chinese hamster ovary cells contain a dihydrofolate reductase with an altered affinity for methotrexate. Biochemistry 19:4321-4327, 1980.
14. HABER DA, BEVERLEY SM, KIELY ML, ET AL. Properties of an altered dihydrofolate reductase encoded by amplified genes in cultured mouse fibroblasts. J Biol Chem 256:9501-9510, 1981.
15. MELERA PW, LEWIS JA, BIEDLER JL, ET AL. Antifolate-resistant Chinese hamster cells. J Biol Chem 255:7024-7028, 1980.
16. SCHIMKE RT. Gene amplification and drug resistance. Sci Am 243:60-69, 1980.
17. NUNBERG JH, KAUFMAN RJ, SCHIMKE RT, ET AL. Amplified dihydrofolate reductase genes are localized to a homogeneously staining region in a single chromosome in a methotrexate-resistant Chinese hamster ovary cell line. Proc Natl Acad Sci USA 75:5553-5556, 1978.
18. DOLNICK BJ, BERENSON RJ, BERTINO JR, ET AL. Correlation of dihydrofolate reductase elevation with gene amplification in a homogeneously staining chromosomal region in L5178Y cells. J Cell Biol 83:394-402, 1979.
19. KAUFMAN RJ, BROWN PC, and SCHIMKE RT. Amplified dihydrofolate reductase genes in unstably methotrexate-resistant cells is associated with double minute chromosomes. Proc Natl Acad Sci USA 76:5669-5673, 1979.
20. KAUFMAN RJ, and SCHIMKE RT. Amplification and loss of dihydrofolate reductase genes in a Chinese hamster ovary cell line. Mol Cell Biol 1:1069-1076, 1981.
21. BROWN PC, BEVERLY SM, and SCHIMKE RT. Relationship of amplified dihydrofolate reductase genes to double minute chromosomes in unstably resistant mouse fibroblast genes. Mol Cell Biol 1:1077-1083, 1981.
22. KAUFMAN RJ, BROWN PC, and SCHIMKE RT. Loss and stabilization of amplified dihydrofolate reductase genes in mouse S-180 cell lines. Mol Cell Biol 1:1084-1093, 1981.
23. O'HARE K, BENOIST C, and BREATHNACH R. Transformation of mouse fibroblasts to methotrexate resistance by a recombinant plasmid expressing a prokaryotic dihydrofolate reductase. Proc Natl Acad Sci USA 78:1527-1530, 1981.
24. CLINE MJ, STANG H, MERCOLA K, ET AL. Gene transfer in intact animals. Nature 284:422-424, 1981.
25. CURT GA, CARNEY DN, COWAN KH, ET AL. Unstable methotrexate resistance in human small-cell carcinoma associated with double minute chromosomes. N Engl J Med 308:199-202, 1983.
26. HORNS RC, DOWER WJ, and SCHIMKE RT. Human dihydrofolate reductase gene amplification after methotrexate treatment. Proc Am Assoc Clin Res 24:280, 1983.
27. JOLIVET J, SCHILSKY RL, BAILEY BD, ET AL. Synthesis, retention, and biological activity of methotrexate polyglutamates in cultured human breast cancer cells. J Clin Invest 70:351-360, 1982.
28. COWAN KH, and JOLIVET J. A novel mechanism of resistance to methotrexate in human breast cancer cells: lack of methotrexate polyglutamate formation. Clin Res 31:508A, 1983.
29. CURT GA, CARNEY DN, JOLIVET J, ET AL. Defective methotrexate polyglutamation: a mechanism of in vivo resistance in human small cell lung cancer. Proc Am Assoc Clin Res 24:283, 1983.
30. HOWELL SB, MANSFIELD SJ, and TAETLE R. Thymidine and hypoxanthine requirements of normal and malignant human cells for protection against methotrexate cytotoxicity. Cancer Res 41:945-951, 1981.
31. BROWMAN GP, and CSULLOG GW. Modification of in vivo methotrexate antitumor effect in L1210 leukemia by induced impairment of purine salvage. Biochem Pharmacol 30:869-871, 1981.
32. COLEMAN CN, STOLLER RG, DRAKE JC, ET AL. Deoxycytidine kinase: properties of the enzyme from human leukemic granulocytes. Blood 46:791-803, 1975.
33. TATTERSALL MNH, GANESHAGUR UK, and HOFFBRAND AV. Mechanism of resistance of human acute leukemia cells to cytosine arabinoside. Br J Haematol 27:39-46, 1974.
34. RICHARDSON VJ, CURT GA, and RYMAN BE. Liposomally trapped ara-CTP to overcome ara-C resistance in a murine lymphoma in vitro. Br J Cancer 45:559-564, 1982.
35. STEWART CD, and BURKE PJ. Cytosine deaminase and the development of resistance to arabinosylcytosine. Nature New Biol 233:109-110, 1971.
36. CHANG P, WIERNIK PH, REICH SD, ET AL. Prediction of response to cytosine arabinoside and daunomycin in acute non-lymphocytic leukemia. *In* Therapy of Acute Leukemias, Proceedings of 2nd International Symposium, Rome, 1977 (Madelli F, ed). Rome, Lombardo-Editore, 1977, pp 148-156.
37. SMYTH JF, ROBINS AB, and LEESE CL. The metabolism of cytosine arabinoside as a predictive test for clinical response to the drug in acute myeloid leukemia. Eur J Cancer 12:567-573, 1976.
38. DESAINT VINCENT BR, and BUTTEN G. Studies on 1-beta-D-arabinofuranosylcytosine-resistant mutants of Chinese hamster fibroblasts. III. Joint resistance to arabinofuranosylcytosine and to thymidine—a semidominant manifestation of deoxycytidine triphosphate pool expansion. Somat Cell Genet 5:67-82, 1979.
39. RUSTUM Y, and PREISLER HD. Correlation between leukemia cell retention of 1-beta-D-arabinosylcytosine-5′-triphosphate and response to therapy. Cancer Res 39:42-49, 1979.
40. WILEY JS, JONES SP, SAWYER WH, ET AL. Cytosine arabinoside influx and nucleoside transport sites in acute leukemia. J Clin Invest 69:479-489, 1982.
41. CIHAK A. Modulation of orotate phosphoribosyltransferase and orotidine 5′-phosphate decarboxylase activities in regenerating rat liver by 5-azacytidine. Colln Czech Chem Commun 39:3782-3792, 1974.
42. DOSKOL J, PACES V, and SORM F. Inhibition of protein synthesis by 5-azacytidine in *E. coli*. Biochem Biophys Acta 145:771-779, 1967.
43. DRAKE JC, STOLLER RG, and CHABNER BA. Characteristics of the enzyme uridine-cytidine kinase isolated from a cultured human cell line. Biochem Pharmacol 26:64-66, 1977.
44. PLAGMANN PGW, BEHRENS M, and ABRAHAM D. Metabolism and cytotoxicity of 5-azacytidine in cultured Novikoff rat hepatoma and P388 mouse leukemia cells and their enhancement by preincubation with pyazofuran. Cancer Res 38:978-989, 1978.
45. VESELY J, CHIAK A, and SORM F. Biochemical mechanisms of drug resistance. IV. Development of resistance to 5-azacytidine and simultaneous depression of pyrimidine metabolism in leukemic mice. Int J Cancer 2:639-646, 1967.
46. REICHARD P, SKOLD O, KLEIN G, ET AL. Studies on resistance against 5-fluorouracil. I. Enzymes of the uracil pathway during development of resistance. Cancer Res 22:235-243, 1962.
47. REYES P, and HALL TC. Synthesis of 5-fluorouridine-5′-phosphate by a pyrimidine phosphoribosyltransferase of mammalian origin. II. Correlation between the tumor levels of the enzymic and 5-fluorouracil-promoted increase in survival of tumor-bearing mice. Biochem Pharmacol 18:2587-2590, 1969.
48. KASBEKER DK, and GREENBERG DM. Studies on tumor resistance to 5-fluorouracil. Cancer Res 23:818-825, 1963.
49. REICHARD P, SKOLD O, and KLEIN G. Possible enzymatic mechanism for development of resistance against 5-fluorouracil in ascites tumors. Nature 183:939-941, 1959.
50. HOUGHTON JA, MARODA SJ, PHILLIPS JO, ET AL. Biochemical de-

terminants of responsiveness to 5-fluorouracil and its derivatives in xenografts of human colorectal adenocarcinomas in mice. Cancer Res 42:144-149, 1982.

51. HEIDELBERGER C, KALDOR G, MUKHERJEE KL, ET AL. Studies on fluorinated pyrimidines. XI. In vitro studies on tumor resistance. Cancer Res 20:903-909, 1960.
52. ROSSANA C, RAO LG, and JOHNSON LF. Thymidylate synthetase overproduction in 5-fluorouracil-resistant mouse fibroblasts. Mol Cell Biol 2:1118-1125, 1982.
53. BASKIN F, CARLIN SC, KRAUS P, ET AL. Experimental chemotherapy of neuroblastoma. II. Increased thymidylate synthetase activity in a 5-fluorodeoxyuridine-resistant variant of mouse neuroblastoma. Mol Pharmacol 11:105-117, 1975.
54. PRIEST DG, LEDFORD BE, and DOIG MT. Increased thymidylate synthetase in 5-fluorodeoxyuridine-resistant cultured hepatoma cells. Biochem Pharmacol 29:1549-1553, 1980.
55. KESSEL D, and WODINSKY I. Thymidine kinase as a determinant of the response to 5-fluoro-2′-deoxyuridine in transplantable murine leukemias. Mol Pharmacol 6:251-254, 1970.
56. CHABNER BA. Pyrimidine antagonists. *In* Pharmacologic Principles of Cancer Treatment (Chabner BA, ed). Philadelphia, W. B. Saunders, 1982, pp 183-212.
57. GOLDENBERG GJ, LEE M, LAM H-YP, ET AL. Evidence for carrier-mediated transport of melphalan by L5178Y lymphoblasts in vitro. Cancer Res 37:755-760, 1977.
58. WOLPERT MK, and RUDDON RW. A study on the mechanisms of resistance to nitrogen mustard (HN_2) in Ehrlich ascites tumor cells: comparison of uptake of HN_2-C^{14} into sensitive and resistant cells. Cancer Res 29:873-877, 1969.
59. BEGLEITER A, LAM H-YP, GROVER J, ET AL. Evidence for active transport of melphalan by two amino acid carriers in L5178Y lymphoblasts in vitro. Cancer Res 39:353-359, 1979.
60. GOLDENBERG GJ, VANSTON CL, and BIHLER I. Transport of nitrogen mustard on the transport carrier for choline in L5178Y lymphoblasts. Science 172:1148-1149, 1971.
61. REDWOOD WR, and COLVIN M. Transport of melphalan by sensitive and resistant L1210 cells. Cancer Res 40:1144-1149, 1980.
62. GOLDENBERG GJ, VANSTON CL, ISRAELS LG, ET AL. Evidence for a transport carrier of nitrogen mustard in nitrogen mustard-sensitive and -resistant L5178Y lymphoblasts. Cancer Res 30:2285-2291, 1970.
63. GOLDENBERG HL. The role of drug transport in resistance to nitrogen mustard and other alkylating agents in L5178Y lymphoblasts. Cancer Res 35:1687, 1975.
64. BEGLEITER A, LAM HYP, and GOLDENBERG GJ. Mechanism of uptake of nitrosourea by L5178Y lymphoblasts in vitro. Cancer Res 37:1022-1027, 1977.
65. HIRONO I. Mechanism of natural and acquired resistance to methyl-bis-(beta-chloroethyl)amine-*N*-oxide in ascites tumors. Gan 52:39-48, 1961.
66. HIRONO I, KACHI H, and OHASHI A. Mechanism of natural and acquired resistance to methyl-bis-(2-chloroethyl)amine-*N*-oxide in ascites tumors II. Gan 53:73-80, 1962.
67. CALCUTT G, and CONNORS TA. Tumor sulfhydryl levels and sensitivity to the nitrogen mustard merophan. Biochem Pharmacol 12:839-845, 1963.
68. GURTOO HL, HIPKENS JH, and SHARMA CD. Role of glutathione in the metabolism-dependent toxicity and chemotherapy of cyclophosphamide. Cancer Res 41:3584-3591, 1981.
69. NATHAN CF, ARRICK BA, MURRAY HW, ET AL. Tumor cell antioxidant defenses. Inhibition of the glutathione redox cycle enhances macrophage-mediated cytolysis. J Exp Med 153:766-782, 1981.
70. SUZUKAKE K, PETRO BJ, and VISTICA DT. Reduction in glutathione content of L-PAM-resistant L1210 cells confers drug sensitivity. Biochem Pharmacol 31:121-124, 1982.
71. SUZUKAKE K, VISTICA BP, and VISTICA DT. Dechlorination of L-phenylalanine mustard by sensitive and resistant tumor cells and its relationship to intracellular glutathione content. Biochem Pharmacol 32:165-167, 1983.
72. SLADEK NE. Bioassay and relative cytotoxic potency of cyclophosphamide metabolites generated in vitro and in vivo. Cancer Res 33:1150-1155, 1973.
73. CONNORS TA, COX PJ, FARMER PB, ET AL. Some studies of the active intermediate formyl in the microsomal metabolism of cyclophosphamide and isophosphamide. Biochem Pharmacol 23:115-119, 1974.
74. BEACH LR, and PALMITER RD. Amplification of the metallothionein-I gene in cadmium-resistant mouse cells. Proc Natl Acad Sci USA 78:2110-2114, 1981.
75. ENDRESEN L, BAKKA A, and RUGSTAD HE. Increased resistance to chlorambucil in cultured cells with a high concentration of cytoplasmic metallothionein. Cancer Res 43:2918-2926, 1983.
76. LAWLEY PD, and BROOKS P. Cytotoxicity of alkylating agents towards sensitive and resistant strains of *Escherichia coli* in relation to extent and mode of alkylation of cellular macromolecules and repair of alkylation lesions in deoxyribonucleic acid. Biochem J 109:433-447, 1968.
77. KOHN KW, STEIGBIGEL NH, and SPEARS CL. Cross linking and repair of DNA in sensitive and resistant strains of *E. coli* treated with nitrogen mustard. Proc Natl Acad Sci USA 53:1154-1161, 1965.
78. CRAWTHORNE AR, and ROBERTS JJ. Mechanism of the cytotoxic action of alkylating agents in mammalian cells and evidence for the removal of alkylated groups from deoxyribonucleic acid. Nature 211:150-153, 1966.
79. PARSONS PG, SMELLIE SG, MANSON LE, ET AL. Properties of human melanoma cells resistant to 5-(3′,3′-dimethyl-1-triazeno)imidazole-4-carboxamido and other methylating agents. Cancer Res 42:1454-1461, 1982.
80. ERICSON LC, LAURENT G, SHARKEY NA, ET AL. DNA crosslinking and monoadduct repair in nitrosourea-treated human tumor cells. Nature 288:727-729, 1980.
81. BEDFORD P, and FOX BW. Repair of DNA interstrand crosslinks after busulphan. A possible mode of resistance. Cancer Chemother Pharmacol 8:3-7, 1982.
82. CARR FJ, and FOX BW. DNA strand breaks and repair synthesis in Yoshida sarcoma cells with differential sensitivities to biofunctional alkylating agents and UV light. Mutat Res 83:233-249, 1981.
83. DROBNIK J, URBANKOVA M, and KREKULOVA A. The effect of *cis*-dichlorodiammineplatinum (II) on *Escherichia coli* B. The role of fil, exv, and hcr markers. Mutat Res 17:13-20, 1973.
84. ZWELLING LA, MICHAELS S, and SCHWARTZ H. DNA crosslinking as an indicator of sensitivity and resistance of mouse L1210 leukemia to *cis*-diamminedichloroplatinum II and L-phenylalanine mustard. Cancer Res 41:640-649, 1981.
85. DUCORE J, ZWELLING L, and KOHN K. DNA intrastrand crosslinking and cytotoxicity in three Burkitt's lymphoma cell lines treated with cisplatin. Proc Am Assoc Cancer Res and ASCO 21:267, 1980.
86. ERIKSON LC, ZWELLING LA, and DUCORE JM. Differential cytotoxicity and crosslinking in normal and transformed human fibroblasts treated with *cis*-diamminedichloroplatinum (II). Cancer Res 41:2791-2794, 1981.
87. MICETICH K, MICHAELS S, JUDE G, ET AL. Mechanism of resistance to *cis*-dichlorodiammineplatinum (I) in a line of L1210 cells. Proc Am Assoc Cancer Res and ASCO 22:252, 1981.
88. BAKKA A, ENDRESEN L, JOHNSON ABS, ET AL. Resistance against *cis*-dichlorodiammineplatinum in cultured cells with a high content of metallothionein. Toxicol Appl Pharmacol 61:215-226, 1981.
89. WILKENSON CR, COX PJ, JONES M, ET AL. Selection of potential second generation platinum compounds. Biochimie 60:851-857, 1978.
90. MCCALLISTER RJ, GILBERT WR, ASHTON DM, ET AL. Pseudofeedback inhibition of purine synthesis by 6-mercaptopurine ribonucleotide and other purine analogs. J Biol Chem 239:1560-1563, 1964.
91. WAY WL, and PARKS RE. Enzymatic synthesis of 5′-phosphate nucleotides of purine analogs. J Biol Chem 221:467-480, 1958.
92. BROCKMAN RW. Mechanisms of resistance to the anticancer agents. Adv Cancer Res 7:129-234, 1963.

93. RASMAN M, LEE MH, CREASEY WA, ET AL. Mechanisms of resistance to 6-thiopurines in human leukemia. Cancer Res 34:1952-1956, 1974.
94. SCHOLAR EM, and CALABRESI P. Increased activity of alkaline phosphatases in leukemia patients resistant to thiopurines. Biochem Pharmacol 28:445-446, 1979.
95. LEE MH, HUANG UM, and SARTORELLI AC. Alkaline phosphatase activities of 6-thiopurine-sensitive and -resistant sublines of sarcoma 180. Cancer Res 38:2413-2418, 1978.
96. LEE MH, HUANG YM, and SARTORELLI AC. Immunological studies on alkaline phosphatases of 6-thiopurine-sensitive and -resistant sublines of sarcoma 180. Cancer Res 38:2419-2423, 1978.
97. LIPPMAN ME. Glucocorticoid binding proteins in acute lymphoblastic leukemia breast cells. J Clin Invest 52:1715-1725, 1973.
98. SIBLEY CH, and THOMPKINS GM. Mechanisms of steroid resistance. Cell 2:221-227, 1974.
99. AMAHEIN JA, MEYER WJ, JONES HW, JR, ET AL. Androgen insensitivity in man; evidence for genetic heterogeneity. Proc Natl Acad Sci USA 73:891-896, 1976.
100. LING V, AUBIN JE, CHASE A, ET AL. Mutants of Chinese hamster ovary (CHO) cells with altered colcemid-binding affinity. Cell 18:423-430, 1979.
101. CABRAL F, SOBEL ME, and GOTTESMAN MM. CHO mutants resistant to colchicine, colcemid or griseofuluin have an altered beta-tubulin. Cell 20:29-36, 1980.
102. KEATES RAB, SARANGI F, and LING V. Structural and functional alterations in microtubule protein from Chinese hamster ovary cell mutants. Proc Natl Acad Sci USA 78:5638-5642, 1981.
103. GUPTA RS. Podophyllotoxin-resistant mutants of Chinese hamster ovary cells: cross-resistance studies with various microtubule inhibitors and podophyllotoxin analogues. Cancer Res 43:505-512, 1983.
104. GUPTA RS, HO TKW, MOFFAT MRK, ET AL. Podophyllotoxin-resistant mutants of Chinese hamster ovary cells. J Biol Chem 257:1071-1078, 1982.
105. MYERS CE. Anthracyclines. *In* Pharmacologic Principles of Cancer Treatment (Chabner BA, ed). Philadelphia, W. B. Saunders, 1982, pp 416-434.
106. KANTER PM, and SCHWARTZ HS. Quantitative models for growth inhibition of human leukemia cells by antitumor anthracycline derivatives. Cancer Res 39:3661-3666, 1979.
107. DUVERNAY VH, PATCHER JA, and CROOKE S. Deoxyribonucleic acid binding sites on several new anthracycline antitumor antibiotics, sequence preference and structure-activity relationships of marcellomycin and its analogs compared to adriamycin. Biochemistry 18:4024-4031, 1979.
108. TOKES ZA, RODGERS KE, and REMBAUM A. Synthesis of adriamycin coupled to polyglutaraldehyde microspheres and the evaluation of their cytostatic activity. Proc Natl Acad Sci USA 79:2026-2030, 1982.
109. RODGERS KE, CARR BI, and TOKES ZA. Cell surface-mediated cytotoxicity of polymer-bound adriamycin against drug-resistant hepatocytes. Cancer Res 43:2741-2748, 1983.
110. TAYLOR RF, TEAGUE LA, and YESAIR DW. Drug-binding macromolecular lipids from L1210 leukemia tumors. Cancer Res 41:4316-4323, 1982.
111. BACHUR NR, and GEE MV. Microsomal reductive glycosidase. J Pharmacol Exp Ther 197:681-686, 1976.
112. AKIYAMA S, and KUWANO M. Isolation and preliminary characterization of bleomycin-resistant mutants from Chinese hamster ovary cells. J Cell Physiol 107:147-153, 1981.
113. AKIYAMA S, IKEZAKI K, KUNAMOCHI H, ET AL. Bleomycin-resistant cells contain increased bleomycin-hydrolase activities. Biochem Biophys Res Commun 101:55-60, 1981.
114. SINHA BK. Binding specificity of chemically and enzymatically activated anthracycline anticancer agents to nucleic acids. Chem Biol Interact 30:67-72, 1980.
115. MUNGIKAR A, CHITNIS M, and GOTHOSKAR B. Mixed function oxidase enzymes in adriamycin-sensitive and -resistant sublines of P388 leukemia. Chem Biol Interact 35:119-124, 1981.
116. BABSON JR, ABELL NS, and REED DJ. Protective role of the glutathione redox cycle against adriamycin-mediated cytotoxicity in isolated hepatocytes. Biochem Pharmacol 30:2299-2304, 1981.
117. DOROSHOW JH, LOCKER GY, and MYERS CE. Enzymatic defenses of the mouse heart against reactive oxygen metabolites, alterations produced by doxorubicin. J Clin Invest 65:128-135, 1980.
118. GANAPATHI R, REITER W, and KRISHAN A. Intracellular adriamycin levels and cytotoxicity in adriamycin-sensitive and adriamycin-resistant P388 mouse leukemia cells. JNCI 68:1027-1032, 1982.
119. BASKIN F, ROSENBERG RN, and DEV V. Correlation of double minute chromosomes with unstable multidrug cross-resistance in uptake mutants of neuroblastoma cells. Proc Natl Acad Sci USA 78:3654-3658, 1981.
120. DANO K. Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. Biochim Biophys Acta 323:1466-1483, 1973.
121. INABA M, KABAYASHI H, SAKURAI Y, ET AL. Active efflux of daunorubicin and adriamycin in sensitive and resistant sublines of P388 leukemia. Cancer Res 39:2200-2203, 1979.
122. KESSEL D, personal communication.
123. HARKER WG, personal communication.
124. KEMPE TD, SWYNYD EA, BRUIST M, ET AL. Stable mutants of mammalian cells that overproduce the first three enzymes of pyrimidine nucleotide biosynthesis. Cell 9:541-550, 1976.
125. HUNT SW, and HOFFEE PA. Increased adenosine deaminase synthesis and messenger RNA activity in deoxycoformycin-resistant cells. J Biol Chem 257:41-44, 1982.
126. KUZIK BA, and WRIGHT JA. Hydroxyurea-resistant mouse L cells with elevated levels of drug-resistant ribonucleotide reductase activity. Biochem Genet 18:311-329, 1980.
127. WILKOFF W, and DULMADGE EA. Resistance and cross-resistance of cultured leukemia P388 cells to vincristine, adriamycin, adriamycin analogs, and actinomycin D. JNCI 61:1521-1524, 1978.
128. KAYE SB, and BODEN JA. Cross-resistance between actinomycin-D, adriamycin, and vincristine in a murine solid tumor in vivo. Biochem Pharmacol 29:1081-1084, 1980.
129. BECH-HANSON NT, TILL JE, and LING V. Pleiotropic phenotype of colchicine-resistant CHO cells: cross-resistance and collateral sensitivity. J Cell Physiol 88:23-32, 1976.
130. LING V, and THOMPSON LH. Reduced permeability in CHO cells as a mechanism of resistance to colchicine. J Cell Physiol 83:103-116, 1973.
131. JULIANO RL, and LING V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152-162, 1976.
132. CARLSEN SA, TILL JE, and LING V. Modulation of drug permeability in Chinese hamster ovary cells. Possible role for phosphorylation of surface glycoproteins. Biochim Biophys Acta 467:238-250, 1977.
133. INABA M, and SAKURAI Y. Enhanced efflux of actinomycin-D, vincristine and vinblastine in adriamycin-resistant subline of P388 leukemia. Cancer Lett 8:111-115, 1979.
134. INABA M, KOBAYASHI H, SAKURAI Y, ET AL. Active efflux of daunorubicin and adriamycin in sensitive and resistant sublines of P388 leukemia. Cancer Res 39:2200-2203, 1979.
135. KESSEL D, and BOSMAN HB. On the characteristics of actinomycin-D resistance in L5178Y cells. Cancer Res 30:2695-2701, 1970.
136. BOSMANN HB. Mechanism of cellular drug resistance. Nature 233:566-569, 1971.
137. BIEDLER JF, and PETERSON PHF. Altered plasma membrane glycoconjugates of Chinese hamster cells with acquired resistance to actinomycin D, daunorubicin and vincristine. *In* Molecular Actions and Targets for Cancer Chemotherapeutic Agents (Sartorelli AC, Lazlo JS, and Bertino JR, eds). New York, Academic Press, 1981, pp 453-482.
138. PETERSON PHF, MYERS MB, SPENGLER BA, ET AL. Alteration of plasma membrane glycoproteins and gangliosides of Chinese hamster ovary cells accompanying development of resistance to daunorubicin and vincristine. Cancer Res 43:222-225, 1983.

139. BECK WT, MUELLER TJ, and TANZER LR. Altered surface membrane glycoproteins in vinca-alkaloid-resistant human leukemic lymphoblasts. Cancer Res 39:2070-2076, 1979.

140. DEBENHAM PG, KARTNER H, SIMONOVITCH L, ET AL. DNA-mediated transfer of multiple drug resistance and plasma membrane glycoprotein expression. Mol Cell Biol 2:881-889, 1982.

141. BECK WT, and ARTAIN MC. Continued expression of vinca alkaloid resistance by CCRF-CEM cells after treatment with tunicamycin or pronase. Cancer Res 42:184-189, 1982.

142. TSURUO T, IIDA H, TSUKAGOSHI S, ET AL. Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res 41:1967-1972, 1981.

143. ______. Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. Cancer Res 42:4730-4733, 1982.

144. SLATER LM, MURRAY SL, and WETZEL MW. Verapamil restoration of daunorubicin responsiveness in daunorubicin-resistant Ehrlich ascites carcinoma. J Clin Invest 70:1131-1134, 1982.

145. INABA M, FUJIKURA R, and SAKURAI Y. Active efflux common to vincristine and daunorubicin in vincristine-resistant P388 leukemia. Biochem Pharmacol 30:1863-1865, 1981.

146. TSURUO T, IIDA H, TSUKAGOSHI S, ET AL. Potentiation of vincristine and adriamycin effects in human hemopoietic tumor cell lines by calcium antagonists and calmodulin inhibitors. Cancer Res 43:2267-2272, 1983.

147. GIAVAZZI R, SCHOLAR E, and HART IR. Isolation and preliminary characterization of an adriamycin-resistant murine fibrosarcoma cell line. Cancer Res 43:2216-2222, 1983.

148. TSURUO T, IIDA H, NOJIRI M, ET AL. Potentiation of chemotherapeutic effect of vincristine in vincristine-resistant tumor-bearing mice by calmodulin inhibitor chloripramine. J Pharm Dyn 6:145-147, 1983.

149. TSURUO T, IIDA H, NOJIRI M, ET AL. Circumvention of vincristine and adriamycin resistance in vitro and in vivo by calcium influx blockers. Cancer Res 43:2905-2910, 1983.

150. INABA M, FUJIKURA R, TSUKAGOSHI S, ET AL. Restored in vitro sensitivity of adriamycin and vincristine-resistant P388 leukemia with reserpine. Biochem Pharmacol 30:2191-2194, 1981.

Regional Cancer Chemotherapy[1]

William D. Ensminger* and John W. Gyves[2,3]

Regional cancer chemotherapy is a means of exploiting dose-response effects by delivering more drug to regionally confined tumors. Regional chemotherapy can be divided into two major categories, third-space (cerebrospinal, peritoneal, pleural, and pericardial fluid) and intra-arterial treatments. Interest in regional chemotherapy has risen recently for a variety of reasons, including evidence for significantly higher response rates compared to systemic therapies. The applicable pharmacokinetic principles for drug selection have been defined and indicate that regional drug exposure advantage is directly proportional to total-body drug clearance and is inversely proportional to rate of egress from a third-space or to regional blood flow. Reliable drug delivery to the entire region in question is important. The recently introduced totally implanted devices (pumps and ports) make regional therapy more reliable, safe, and convenient. In addition, nuclear medicine scanning techniques have proven useful in assessing drug distribution with regional delivery. Regional therapy may control regional tumor, but extraregional failure may ensue. However, when regional therapy is regionally selective in its toxic effects, systemic therapy may be combined in full doses. Future efforts will need to focus on the development of more potent regional therapies, alone and in combination with systemic therapies, as well as on the validation of such treatments through controlled clinical trials. **[Cancer Treat Rep 68:101–115, 1984]**

PERSPECTIVE ON REGIONAL CANCER CHEMOTHERAPY

A number of common neoplasms have a propensity for confinement to specific body regions or organs. Frequently, it is the regional tumor, not widespread metastases, which is the primary cause of morbidity and, eventually, death. In a few circumstances, surgical resection of an isolated metastasis can be curative. For example, resection of a single, isolated tumor nodule metastatic to the liver can lead to a cure in up to 25% of cases (1). Unfortunately, even regionally confined metastases are seldom single and are often surgically unresectable because of anatomic location. When all metastatic tumor can be encompassed in a radiation port with sufficient sparing of vital normal tissues, high-dose radiotherapy can sterilize such disease. However, normal tissue tolerance to radiotherapy often impedes the delivery of adequate dosages. Regional chemotherapy represents a third potent approach that is useful in regionally confined cancers when resection and/or radiation therapy cannot produce local control or cure.

Regional chemotherapy is based on the premise that many chemotherapeutic agents display a steep dose response for toxicity and for therapeutic effect (2). Even in circumstances where systemically administered (iv) chemotherapy is relatively ineffective, regional chemotherapy may still improve the likelihood of response by the generation of much greater drug exposures (higher levels, longer times). Regional chemotherapy administration represents a means to generate increased drug exposure in the region where the tumor resides, while maintaining a lower drug exposure at the level of dose-limiting normal host tissues elsewhere in the body. Ideally, the higher level of drug generated regionally will be cytotoxic to the regionally confined tumor, while the lower systemic drug level will be below the toxic threshold for the usual dose-limiting tissues, such as bone marrow and gastrointestinal mucosa. With sufficient regional selectivity, dose-limiting toxicity should be manifested by the normal tissues of the region infused and not by tissues elsewhere in the body. In this regard, regional chemotherapy has similarities to radiation therapy, but may be more selective in those situations where tumor and normal tissue in the treated region differ significantly in intrinsic drug sensitivity.

---

[1] Supported in part by Public Health Service grants CA-28478, CA-28490, and CA-33825 (National Cancer Institute), and M01-RR-00042 (Division of Research Resources), National Institutes of Health, Department of Health and Human Services, and by the Burroughs Wellcome Fund.

[2] Cancer Pharmacology Program, University of Michigan Medical School, Upjohn Center for Clinical Pharmacology, Ann Arbor.

[3] The collaboration of John Niederhuber (Oncologic Surgery), William Chandler (Neurosurgery), and Harry Greenberg (Neurology), University of Michigan, has been essential to our research on regional chemotherapy. We would also like to recognize the technical assistance of P. Stetson, M. Janis, S. Walker, S. Gilbertson, C. Knutsen, R. Castello, and B. Castello.

**Reprint requests to:* William D. Ensminger, PhD, MD, Upjohn Center for Clinical Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109.

As will be described below, regional drug exposures may be increased 100- to 1000-fold/given degree of systemic exposure. Such selective improvements are due to the pharmacokinetics of individual agents and to restricted egress from regional compartments or to circumscribed, relatively low regional blood flows. Despite improved regional drug exposure, the antineoplastic effect may not be improved if a tumor is either too sensitive or too refractory. If a tumor is extremely sensitive to a given drug, systemic administration may suffice to achieve maximum therapeutic effect. If a tumor is totally refractory to all concentrations of a drug, then the increased exposure generated through regional chemotherapy will be of no benefit. Unfortunately, little dose response data exist for many of the drugs and tumors that are clinically significant. In a sense, regional chemotherapy trials may contribute to such knowledge by determining response rates and the degree of tumor cytoreduction at the higher drug exposure generated regionally.

Regional chemotherapy can be divided into two major categories: administration into a third-space regional compartment (cerebrospinal fluid [CSF] space, peritoneal cavity, pleural space, and pericardial space) and intra-arterial infusion into the afferent artery feeding the tumor-containing organ or body region. The principles involved are broadly applicable within each category and, therefore, only the most frequent and significant regional chemotherapies will be discussed below.

In a real sense, interest in regional chemotherapy stems from the relative lack of efficacy of systemic chemotherapy in the treatment of a number of common tumors which display confinement to specific body regions. Although few adequately controlled studies have been performed, response rates for regional chemotherapy in the treatment of regional tumors have generally been higher than response rates seen with systemic chemotherapy.

Two recent developments in clinical pharmacology have proved to be a major impetus to regional chemotherapy. The first development has been the theoretic and practical exposition of the pharmacokinetics relevant to regional chemotherapy. It is possible to measure drugs readily in body fluids and, although a number of techniques have been utilized, high-performance liquid chromatographic assays have been developed for most antineoplastic agents of interest. Hence, the basic pharmacokinetic properties relevant to a drug's potential usefulness in regional chemotherapy are often determined early in clinical protocols. When a given drug has the appropriate properties, the improved regionally selective drug levels can be directly measured in clinical practice. The dependence of selectivity on route of administration, dose rate, schedule, infusion volumes, etc, can be ascertained by comparing the regional and systemic drug levels achieved with regional versus systemic administration.

The second impetus to regional chemotherapy has been the development of improved devices for the controlled delivery of drug solutions into appropriate blood vessels or body spaces. Extracorporeal volumetric infusion pumps have improved in accuracy and reliability. With refinements in drug therapy and more potent drugs, such external infusion pumps have proliferated in number within the hospital setting. Portable external pumps have come into use and are being refined. However, the development of a totally implanted pump (3-5) has made it possible to give protracted infusions safely and reliably to outpatients, with relatively few restrictions on the patient's lifestyle. The only commercially available pump, the Model 400 Infusaid Pump (Infusaid Corp, Norwood, MA), has been implanted in several thousand patients. Although most pumps implanted have been for hepatic arterial chemotherapy, some have been used for intrathecal morphine infusions for pain control (6), for intraventricular and intra-arterial treatment of central nervous system (CNS) tumors (7,8), for iv infusions of heparin in thrombotic disorders (9), and for iv infusions of insulin in Type II diabetes (10). Externally programmable. yet totally implantable pumps produced by a number of manufacturers are in early clinical trials (11). Implantable infusion pumps are not suitable for drugs requiring administration in large fluid volumes, for drugs that are unstable, and for drugs best administered in boluses or short infusions. However, even for such drugs and, in circumstances where reliable repeated access is required, small implanted injection ports attached to silastic catheters can provide a safe and convenient means of access (12).

THIRD-SPACE CHEMOTHERAPY

There are a number of circumstances where tumors are largely confined to body cavities (or third spaces) without significant invasion into lining tissues. Notable examples are meningeal carcinomatosis in the CSF space and malignant ascites within the peritoneal cavity. Direct drug instillation into the CSF space has been frequently practiced for control of meningeal tumor, most commonly using methotrexate (MTX)

(13,14). In the past few years, direct drug administration into the peritoneal cavity has also been extensively investigated (15-17). The pharmacologic principles applicable to these two spaces have similarities and can be generalized to other body spaces such as the pleural and pericardial cavities (18).

Third-Space Pharmacokinetic Principles

The pharmacokinetic properties of a given drug determine whether direct administration into a given body space will be advantageous in terms of generating increased drug exposure in the fluid (and on the surfaces lining that space) relative to systemic exposure. The pharmacokinetic modeling appropriate to antineoplastic drugs for third-space administration has been defined (15,18). A drug within the body cavity crosses the lining membrane of area (A) by a diffusive process characterized by a permeability (P). As commonly practiced, the intrinsic P is not measured separately, but is measured only in combination with the associated A to generate a permeability-area product (PA).

CSF Space

Using a simplified two-compartment model (19), the mass balance equation is

$$-V_{CNS}\,\frac{dC_{CSF}}{dt} = r + PA\,(C_{CSF} - C_p) + Q_{CSF}\,C_{CSF}, \qquad [1]$$

where V_{CNS} equals the volume of CSF plus the extracellular fluid volume of the brain and spinal cord, C_{CSF} equals the CSF drug concentration, C_p equals the plasma drug concentration, Q_{CSF} equals the rate of bulk flow of CSF from the CSF space by arachnoid villi uptake, and r equals the active transport component (r is felt to be 0 for MTX). For MTX, PA is approximately 1 ml/hour with a CSF bulk flow (Q_{CSF}) of approximately 30 ml/hour or 0.5 ml/minute (19). Hence, bulk flow dominates the removal of MTX from the CSF space. We feel that the above analysis can be simplified in the case of MTX, since it is not metabolized in the CSF space. A direct constant infusion of MTX into the CSF space at rate I should lead to a steady-state infusion into the vascular compartment (or systemic circulation), which is also at rate I. The resultant systemic plasma level (C_p) is related to the infusion rate and total-body clearance (CL_{TB}) as follows:

$$C_p = I/CL_{TB}. \qquad [2]$$

If the steady-state dose rate of MTX infusion into CSF space is much larger than the quantity of drug transported from plasma back to CSF space, then

$$C_{CSF} \cong I/Q_{CSF} \qquad [3]$$

and,

$$\text{Selective advantage} = C_{CSF}/C_p \cong CL_{TB}/Q_{CSF}. \qquad [4]$$

For MTX, which is excreted primarily by the kidneys, CL_{TB} equals or exceeds creatinine clearance and is thus usually at least 100 ml/minute. Hence, with direct CSF space infusion of MTX,

$$\text{Selective advantage} \cong \frac{CL_{TB}}{Q_{CSF}} \cong \frac{100\ \text{ml/minute}}{0.5\ \text{ml/minute}} \cong 200. \qquad [5]$$

A totally implanted system for direct constant intraventricular infusions has been developed in monkeys and utilized in patients with CNS tumors (7). Using this system in patients, the selective advantage at steady state for direct intraventricular infusion of MTX is ≥ 200, which is as predicted above. Assuming that the bulk flow rate, Q_{CSF}, is relatively constant at approximately 0.5 ml/minute, then CL_{TB} becomes the crucial factor in determining the selective regional advantage of MTX, and probably of many other drugs. It will be seen later that a drug's total-body clearance is a crucial factor in the determination of a drug's selectivity with infusions into other body cavities, as well as with regional intra-arterial infusions.

Floxuridine (FUDR) and cytarabine (AraC) are two pyrimidine antimetabolites which have an even higher CL_{TB} than MTX. Constant intraventricular infusion of FUDR, in particular, generates at least a 1000-fold higher steady-state CSF space level than plasma level (20). The PAs for FUDR and AraC have not been determined, but are probably greater than that for MTX, based on their CSF pharmacokinetics in comparison with ytterbium-169-labeled diethyltriamine pentacetic acid (DTPA), an agent which is removed from the CSF space solely by bulk flow.

Although a system for constant CSF-space drug infusion has recently been developed, the vast majority of prior investigational clinical uses have concerned bolus drug injections into the CSF space (14). The development of ventriculostomy reservoirs has greatly facilitated repeated access to the entire CSF space. Injection of a drug solution into a lateral ventricle leads to distribution throughout the CSF space, as most CSF is produced by the ventricular choroid plexus, whereas injection of drug into the lumbar sac does not always lead to adequate retrograde drug flow into the ventricles (13,21). With bolus intraventricular injection of MTX, CSF drug levels are generated which are several hundred-fold concurrent systemic levels (13). MTX levels are not uniform throughout the CSF space at any given time-point, due to bulk flow of CSF from the site of injection with continued CSF production. Systemic plasma levels rise and peak over 4-8 hours with the arrival of the wave of MTX-containing CSF at the arachnoid villi. Thereafter, both CSF and plasma levels fall in a relatively parallel fashion with time. The systemic plasma levels achieved with bolus intraventricular injection may exceed the toxic threshold for a long enough time to generate systemic toxicity with MTX. Frequent intraventricular injection of much smaller MTX doses has been proposed as a mechanism to sustain therapeutic CSF-space drug levels, without the generation of toxic systemic levels (22). In that regard, constant intraventricular infusion is likely to offer the best method for precise maintenance of a high CSF level with a lower systemic level. Other factors, such as cost and risks of an implanted system, as well as duration of need for therapy, will play an important role in determining whether to implant a pump for constant infusion or to simulate a constant infusion with multiple daily injections. With antimetabolites such as MTX, AraC, and FUDR, cytotoxicity is generally related to duration of exposure above a threshold concentration, with the toxic threshold and duration variable from tissue to tissue (23). These antimetabolites are cell-cycle and phase-specific, which means that nonproliferating cells, such as normal brain and stromal tissues, are less sensitive than proliferating tumor cells. The longer the drug exposure is maintained above the toxic threshold for the tumor cells in the CSF space, the greater is the proportion of tumor cells which should be destroyed on this cytokinetic basis.

The only other antineoplastic agent used intrathecally to any extent has been the alkylating agent thiotepa (24). Thiotepa is more lipid-soluble than the aforementioned antimetabolites and, therefore, is likely to be more readily absorbed (ie, have a larger PA) from the CSF space than MTX, AraC, and FUDR. It is noteworthy that alkylating agents usually generate cytotoxicity more in relation to their peak level than for duration of exposure. Hence, bolus administration of intraventricular thiotepa may be the most rational schedule.

Intraperitoneal and Other Third Spaces

The pharmacokinetic rationale for the ip administration of antineoplastic agents has been delineated by Dedrick et al (15) at the National Cancer Institute and generalized to pleural and pericardial administration of MTX by Howell et al (18). At steady state, the selective advantage of a direct drug infusion into a third space in terms of third-space level (C_{TS}) to systemic plasma level (C_p) relates as follows to the total-body drug clearance (CL_{TB}) and the PA:

$$\text{Selective advantage} = \frac{C_{TS}}{C_p} = \frac{R(PA) + CL_{TB}}{PA}, \qquad [6]$$

where R represents the effusion to plasma MTX concentration partition ratio determined by equilibrium dialysis (required to account for plasma protein binding). When R equals 1 as it does for MTX, then:

$$\text{Selective advantage} = \frac{C_{TS}}{C_p} = 1 + \frac{CL_{TB}}{PA}. \qquad [7]$$

Table 1 summarizes the data obtained by several investigators pertaining to constant infusion of ip MTX and fluorouracil (FU) and of intrapleural and intrapericardial MTX. Thus, it can be seen that for both MTX and FU, direct infusion into a third space usually generates at least a 100-fold selective third-space drug exposure and that this exposure is a function of CL_{TB} and the PA for the drug. If CL_{TB} falls, the selective advantage may fall with constant PA; hence, the importance of sustaining a high CL_{TB} through maintenance of adequate hepatic and renal functions. If the membrane area for diffusion decreases, then PA will fall and, for constant CL_{TB}, the selective advantage for the space defined by the membrane in question will rise. Table 2 summarizes data on short-term, 4-6-hour ip instillations of FU, MTX, doxorubicin, and cisplatin. It can be seen that the selectivity, in terms of higher peritoneal levels and exposure, ranges from tenfold to several hundred-fold, depending on the agent. As the above formula indicates, the ratio of total-body clearance to the permeability area product is indicative of the selectivity achieved.

Intracavitary chemotherapy is most applicable to the treatment of free-floating tumor cells and of tumors of small thickness on the surface lining the cavity. Grossly visible tumors are likely to receive their blood supply

TABLE 1.—Selectivity of direct constant drug infusions into third spaces

Space	Drug	Concurrent drug levels (μM)		C_{TS}/C_p	Ref No.
		Third-space (C_{TS})	Plasma (C_p)		
CSF	MTX	2-30	< 0.01	200-3000	7
CSF	FUDR	4-14	< 0.05	⩾ 1000	20
Peritoneal	FU	100-2000	0.10-0.46	200-8000	25
Peritoneal	MTX	24	0.7	30	18
Pleural	MTX	213	1.0	200	18
Pericardial	MTX	434	1.2	300	18

from the systemic circulation, with limited drug diffusion from the surface facing the cavity. Intracavitary treatment is most likely to be effective against microscopic tumor, yet there is difficulty in quantitating response under that circumstance. The loss of malignant cytology in an effusion has been used by some to denote a response. However, such a response can be meaningful only when there are no gross tumors and when the entire surface lining the cavity is being bathed by the drug-containing solution (ie, there should be no loculated sanctuaries with limited access to the drug solution). The two techniques used to ascertain fluid distribution within a cavity, nuclear scanning with intracavitary ^{99m}TC sulfur colloid and computerized tomography after intracavitary contrast administration, have limited abilities to assure complete distribution within the peritoneal cavity.

Interest has been stimulated in the use of ip chemotherapy for the treatment of ovarian cancer, particularly in an adjuvant setting (15). Ovarian cancer spreads intra-abdominally within peritoneal fluid. Single cells implant on the peritoneal surface, exfoliate, and migrate via the ascitic fluid to new sites. Application of ip therapy can expose individual floating tumor cells and microscopic surface implants to much higher drug levels than achievable with systemic infusion. Such high drug levels may directly kill tumor cells but, in addition, may change the peritoneal surface so that implantation becomes less likely (a poor soil hypothesis). To date, the results of these efforts are preliminary, but suggest that ip chemotherapy may be effective in adjuvant therapy for early stages of ovarian cancer (27).

About 70% of FU given ip exits via the portal vein (26). With ip FU administration, portal vein drug levels are about fourfold higher than levels generated by iv administration of FU at the same dose rate. In addition, it is likely that the lymphatic drainage from the peritoneal cavity contains high drug concentrations with ip FU administration. As further discussed below, increased ip drug exposure and portal vein FU levels with ip FU administration may be advantageous in the adjuvant treatment of high-risk colon cancer.

For hepatic tumors, it is likely that the original microscopic (metastatic) tumor cell deposits derive much of their blood supply from the portal vein (30). Hence, for adjuvant treatment of microscopic liver metastases, portal vein infusions or ip drug infusions may be rational. However, with the neovascularization accompanying tumor growth, the portal supply is obliterated and the arterial supply becomes predominant for grossly detectable disease (31).

Without adjuvant treatment, primary resection of colon cancer which has penetrated through the bowel wall to the serosa and adjacent lymph nodes (Dukes C) leads to cure in only about 25% of patients. In one British study, constant infusion of FU (1 g/day) into the portal vein for 7 days postoperatively after primary resection of Dukes C lesions led to a reduction in the relapse rate at 2 years from 68% to 17% (32). Direct ip FU administration could potentially achieve the same end as a portal vein infusion. Direct portal vein infusion of FU (assuming a portal vein blood flow of 500 ml/minute and a CL$_{TB}$ of FU of 5 L/minute [*see* discussion below]) should generate a 100-fold increased hepatic exposure, as compared to the fourfold increase in portal vein FU level anticipated with ip administration. Currently, adjuvant studies are under way at the National Cancer Institute and elsewhere using ip FU for high-risk colon cancer.

Apropos of the British study above, the contribution of starting the 7-day FU infusion immediately postoperatively in the prevention of tumor implantation and growth throughout the body versus the contribution of higher portal FU levels in killing microscopic intrahepatic implants needs to be ascertained. The physiologic milieu postoperatively may favor implantation of tumor cells, and perhaps even a systemic FU

TABLE 2.—Regional pharmacokinetic selectivity for FU, MTX, doxorubicin, and cisplatin given ip (using a 4- or 6-hr dwell time)

Drug	Peritoneal peak concentration	Systemic peak concentration	Selectivity[*]		CL[†]	PA[‡]	CL/PA	Ref No.
			Peak	AUC				
FU (1040 mg/2 L)	1100 μM	11 μM	100	367	7800	21	371	16,26
MTX	~20 μM	1 μM	~20		135	8	17	28,29
Doxorubicin (60 mg/2 L)	56 μM	0.13 μM	430					27
Cisplatin								
90 mg/m^2/2 L	40 μg/ml	1.6 μg/ml	25	13	450	40	11	17
270 mg/m^2/2 L	85 μg/ml	7.5 μg/ml	11		390	60	6.5	17

[*] Peritoneal value/systemic value; AUC = area under concentration-time curve.

[†] Apparent total-body clearance (ml/min).

[‡] Permeability area product (ml/min).

infusion would inhibit such implantation if given immediately after operation. It is an accepted principle/phenomenon in adjuvant chemotherapy that treatment results are markedly affected by delay in giving drug postoperatively. Recently introduced drug delivery devices (external and implantable pumps and injection ports) may make randomized trials of portal vein, ip, and systemic adjuvant treatment with constant-infusion FU and other drugs more feasible.

INTRA-ARTERIAL CHEMOTHERAPY

Of all the forms of regional chemotherapy practiced, experience has been greatest with intra-arterial therapy. Intra-arterial chemotherapy has been most extensively applied to the treatment of primary and metastatic cancer in the liver, with a history going back > 20 years. However, other body regions and tumor types studied in the past and currently under investigation include external carotid arterial infusions for head and neck cancers, internal carotid and vertebral arterial infusions for primary and metastatic brain tumors, femoral and brachial arterial infusions for melanomas and sarcomas of the extremities, and infusions of various pelvic arteries for urogenital tumors.

There are a number of crucial elements in successful intra-arterial chemotherapy: (*a*) use of a drug which has appropriate pharmacokinetics, dose-response, and schedule dependency; (*b*) catheter placement to infuse the entire tumor-bearing region; and (*c*) use of a drug delivery system which allows repeated and long-term administration conveniently, reliably, and safely, preferably in an outpatient setting.

Pharmacokinetics

Pharmacokinetic analyses outlining the crucial elements and potential drug exposure increase with intra-arterial drug infusions have been carried out by Eckman et al (33), Chen and Gross (34), and Collins and Dedrick (35). To quantitate the delivery advantage to the site in question, the ratio of regional exposure after regional arterial infusion is compared to that after venous infusion at the same dose rate. The advantage of increased drug delivery resulting from arterial infusion is determined by factors which influence the time integrals of the arterial drug levels obtained by arterial versus venous infusion, respectively. Using a physiologically based pharmacokinetic model, Chen and Gross (34) demonstrated that the regional drug exposure advantage (R_d) for agents with linear pharmacokinetics is related to the rate of drug elimination in the rest of the body and the blood perfusion rate into the target region by the formula

$$\text{Advantage } (R_d) = 1 + CL_{app}/Q_T, \qquad [8]$$

where CL_{app} is the total-body drug clearance from the rest of the body, excluding the tumor (target) region, and Q_T is the blood flow through the regional artery being infused. Intra-arterial infusion will reduce drug delivery and availability to the rest of the body by

$$R_d = 1 - E_T, \qquad [9]$$

where E_T is the extraction ratio of the drug by the target region. Combining the advantage gained through the total-body clearance relative to regional blood flow plus that gained by regional extraction, the general equation defining the regional advantage of intra-arterial infusion is:

$$\text{Regional advantage} = 1 + \frac{CL_{TB}}{Q_T\,(1-E_T)}, \qquad [10]$$

where CL_{TB} is total-body drug clearance and Q_T and E_T are as noted above (35). Hence, in analogy with third-space drug infusions (equations [6] and [7]), regional selectivity is again a direct function of a drug's total-body clearance. Thus, agents with high total-body clearance are essential for maximum regional effect for both third-space and intra-arterial chemotherapy.

Table 3 presents, for a number of pertinent drugs, the relevant pharmacokinetic parameters and estimates of the improved regional hepatic exposure with hepatic-arterial drug infusion. Most of the drugs used in hepatic-arterial chemotherapy have short plasma half-lives and high total-body clearances. The two most commonly utilized drugs, FU and FUDR, have half-lives of ≤ 10 minutes with high total-body clearances plus extensive hepatic extraction. Calculations based on direct blood level determinations indicate as much as a 100-fold advantage for FU (36) and a 400-fold advantage for FUDR (37) of hepatic-arterial over iv infusion in the generation of an improved hepatic drug exposure. Although the data are limited, significantly (ie, greater than twofold) increased exposure with hepatic-arterial infusion is seen for a number of other agents, including dichloromethotrexate (41), bischlorethylnitrosourea (38), mitomycin (39,42), cisplatin (40), and thymidine (43).

The theoretic principles described above assume that the rate coefficients of drug loss remain constant over the complete range of drug concentrations and throughout the time periods involved. Data obtained with increasing dose rates of thymidine given into the hepatic artery demonstrate that extraction processes can become increasingly saturated with the hepatic extraction dropping from 80% at 4 g/m^2/day to < 20% at 128 g/m^2/day (43). Nonlinear pharmacokinetics with saturable elimination also apparently hold for infusions of the radiosensitizer 5-bromo-2′-deoxyuridine (BUDR) (44). Recent investigation on the dose-rate dependence of hepatic-arterial FU pharmacokinetics (36) has con-

TABLE 3.—Pharmacokinetic properties of drugs used for hepatic-arterial infusion

Drug	T½ (min)	CL_{TB}*	E_H†	Estimated increased exposure of hepatic-arterial infusion‡	Ref No.
FU	10	2-5	0.80	50-100	36
FUDR	< 10	5-15	0.69-0.92	100-400	37
BCNU	< 5	2.5		7-13	38
Mitomycin	≤ 10	0.6	0.07-0.18	3-5	39,42
Cisplatin	20-30	0.3-0.5		2-4	17,40
Dichloromethotrexate		0.2-0.4	0.40-0.70	3-6	41

*Estimated total-body clearance in L/min.

†Hepatic extraction ratio.

‡Assuming an average hepatic-arterial flow rate (Q) of 200-400 ml/min.

firmed the nonlinear pharmacokinetics of FU first described by Collins et al (45) for iv and ip FU administration. At dose rates of 20 mg/kg/day as a constant infusion, the CL_{TB} of FU is approximately 2 L/minute, falling to <0.5 L/minute at dose rates >270 mg/kg/day. Not only does CL_{TB} fall, but the hepatic extraction ratio falls concurrently (from 0.8 to as low as 0.1), so that at high dose rates given into the hepatic artery, much of the regional specificity is lost (36,37). This means that bolus or short, high dose-rate hepatic-arterial infusions of FU will have markedly decreased regional selectivity. High dose-rate infusions of FUDR, on the other hand, appear to retain their regional hepatic selectivity (37).

The administration of a second agent can decrease the total-body clearance and make a given agent less advantageous regionally. For example, the administration of thymidine with FU can decrease the clearance of FU greatly and virtually eliminate any selectivity for hepatic-arterial FU (46).

As the equations above indicate, the concentration of drug in the arterial watershed is dependent on the blood flow rate through the infused artery (assuming high total-body drug clearance). For example, decreasing the blood flow rate by 90% should increase the arterial concentration of drug at any given dose rate by about tenfold. There are at least five methods to decrease arterial blood flow. Note that as arterial blood flow is decreased, it may become difficult to dissociate therapeutic effects because of resultant ischemia from therapeutic effects due to increased drug exposure to the tumor. The five methods for decreasing arterial blood flow are best illustrated as applied to hepatic-arterial infusion.

Ligation of the hepatic artery with placement of a catheter in the distal arterial stump is one method to simultaneously decrease blood flow to the tumor, while exposing the tumor to higher drug concentrations (47,48). In general, the results of this maneuver are, at best, questionably advantageous over intra-arterial drug infusion alone. Rapid collateralization from a multiplicity of vessels, as well as thrombosis in the stump around the catheter, diverts drug flow to only a small portion of the liver. The application of low-flow nuclide angiography using injection of a lung-scanning agent (^{99m}Tc macroaggregated albumin [TcMAA]) through the catheter in such studies can document the drug flow distribution, as well as thrombus formation (5,49,50). Ligation has the added drawback of being irreversible. Recently, a method using operatively positioned strangulation slings on the hepatic artery has been examined as a means to produce transient occlusion of the hepatic artery for up to 16 hours (51). Intra-arterial FU was given concurrently in some of the patients undergoing transient hepatic-arterial occlusion with this method. The procedure apparently was well-tolerated and permanent arterial thrombosis due to the temporary occlusion did not occur.

A second method, usually inadvertent, of decreasing hepatic-arterial blood flow comes with clot formation around the indwelling catheter. Clot formation may decrease blood flow dramatically prior to total occlusion. This should generate significantly increased drug levels in the infused watershed as the formulas above would suggest. Such clot formation is frequent with the use of percutaneous angiographic catheters (52). One recent study noted an improved response rate in those patients where hepatic-arterial occlusion occurred at some point in their therapy (53). This method of decreasing the hepatic-arterial blood flow is essentially uncontrolled and irreversible. Partial occlusion proceeds to total occlusion haphazardly. Once there is total occlusion, no further hepatic-arterial chemotherapy is possible, even in patients where a response has occurred. With total occlusion, collateralization occurs rapidly so that tumor is still well-nourished from a multiplicity of alternate inaccessible arterial sources (54). Such patients will usually die of progressive hepatic tumor once the therapeutic effect of thrombosis has been lost.

A third method for temporary reduction of hepatic-arterial blood flow has been the use of balloon catheters (55,56). Important factors to be considered are the degree of occlusion (determined by vessel size and the degree of balloon inflation) and the duration of the blood flow reduction induced by balloon inflation. If the reduction of flow can be controlled and quantitated, balloon catheters might prove very useful for drug regimens where short infusions or bolus injections are given. Additional considerations with such regimens are the appropriateness of the drug-schedule (*see* below) and the flow distribution changes brought about by reducing overall hepatic-arterial blood flow.

A fourth method for reducing hepatic-arterial blood flow has been the infusion of vasoconstricting agents such as epinephrine, angiotensin, or vasopressin. Such agents have been used extensively in pharmacoangiography to better delineate tumor vessels (57). As will be discussed later, the lack of responsive smooth muscle fibers in the wall of tumor vessels provides at least the potential for redirecting blood flow from normal liver (responsive arterioles) to tumor (nonresponsive arterioles) (58). Additionally, vasoconstricting agents may have great impact in reducing overall hepatic arterial flow. Direct arterial infusion of appropriate concentrations of epinephrine or vasopressin may confine the vasoconstriction to the hepatic arterial watershed. In our experience, as little as 1 μg of epinephrine given into the hepatic artery can totally block the hepatic-arterial blood flow in some patients for as long as 5 minutes, without affecting baseline pulse and blood pressure. Contingent on the pharmacokinetic principles delineated previously, concurrent cytotoxic drug administration while blood flow is reduced should generate higher arterial drug levels and, thus, a greater regional tumor exposure. It should be noted that when vasoconstrictors decrease overall flow to the normal liver, systemic drug exposure may actually increase for agents with significant hepatic extraction (59). Thus, studies involving concurrent vasoconstrictors and cytotoxic agents will need extensive monitoring to assure appropriate dose schedules for maximal tumor exposure without loss of regional selectivity due to decreased hepatic extraction.

A fifth method for decreasing hepatic-arterial blood flow is the use of microparticulates or microspheres. At sufficiently high doses, approximating 90 million biodegradable starch microspheres (40-μm diameter), hepatic-arterial blood flow can be totally blocked in about 25% of patients (60-62). By 30 minutes after hepatic-arterial injection, the starch microspheres are completely lysed by serum amylase and flow resumes through the hepatic-arterial tree as ascertained by contrast angiograms. In the remaining 75% of patients, hepatic-arterial flow decreases by 80% and arterial-venous shunting occurs. As will be described below, the use of the hepatic-arterial starch microspheres may be an additional method to deliver more drug to tumors within the liver.

Tumor Vasculature and Blood Flow

Based on the radiographic appearances using contrast angiography, it has been customary to regard tumors as being either hypo- or hyper-vascular relative to normal liver. As tumor nodules grow, the evoked new capillary bed develops at the periphery, so that the most vascularized area is the outer shell of the nodule (63). Although the central core of many tumor nodules in the liver is hypovascular, the periphery of the tumor nodule is generally hypervascular relative to normal liver, as demonstrated by nuclear tomographic scans after hepatic-arterial injection of TcMAA (64,65). The microvascular pattern is consistent with the distribution of tumor cell viability and growth, the central core of the tumor often being necrotic while the peripheral rim of actively proliferating tumor cells has an excellent blood supply (63,66). The presence or absence of a hypovascular core appears to relate more to the size of the tumor nodule than to tumor type (65). Nodules < 8 cm in diameter are uniformly hypervascular, whereas those > 9 cm in diameter display a hypovascular core and a hypervascular rim as ascertained by radionuclide tomographic angiography (table 4). The density of vessels in the hypervascular regions of tumor nodules appears to be twofold to sixfold greater than in normal liver (table 4; fig 1). These observations suggest a number of methods whereby tumor hypervascularity can be used for selective therapeutic advantage.

Tumor vessels tend to lack the smooth muscle layer found in normal precapillary arterioles, and thus are less sensitive to vasoconstricting agents such as epinephrine or vasopressin (58,67). If a relative redistribution of blood flow occurs with vasoconstricting agents, it may provide a means for further selective advantage in the delivery of therapeutic microspheres to hypervascular tumor. Microspheres of 40-80-μm diameter, when injected as a homogeneous suspension into the hepatic artery, should lodge in the hepatic-arterial microvasculature in direct proportion to regional blood flow throughout that watershed. As mentioned earlier, the hepatic-arterial injection of TcMAA (lung scanning agent) with nuclear tomography provides a means to determine the relative blood flow distribution between normal liver and tumor nodules within liver and, thus, to monitor selective delivery of therapeutic microspheres to tumor (64).

Therapeutic (Drug and Radiation) Microspheres

Three varieties of microspheres have been used clinically, two for drug delivery and one for radiotherapy. Ethylcellulose microspheres which are 225 ± 55 μm in diameter and contain 80% by weight of biologically active mitomycin have undergone recent testing in Japan (68,69). Preclinical investigations in dogs indicate that drug release occurs over hours and that there is approximately a 60% reduction in circulating systemic mito-

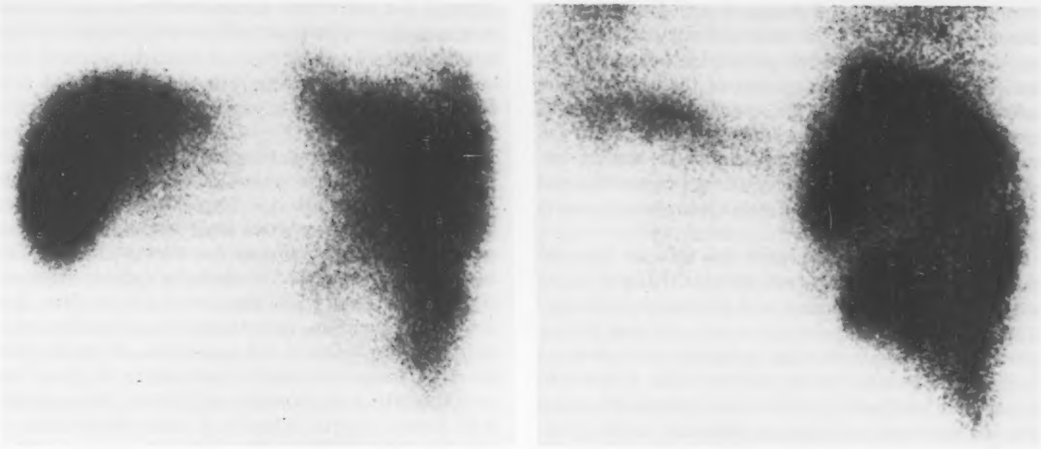


FIGURE 1.—Hepatic tumor hypervascularity seen by TcMAA infusion scan. Posterior view of Tc-sulfur colloid scan (left) shows photon-deficient filling defects corresponding to dense areas of photon excess on a comparable view of a TcMAA infusion scan (right), denoting increased blood flow to tumor relative to normal liver.

mycin when the drug is encapsulated in microspheres and administered intra-arterially (68). In clinical studies, minor responses were seen in a small number of patients with regional tumors (69). Ethylcellulose microspheres are not biogradable and, thus, should permanently occlude the vessels in which they lodge. Hence, the potential therapeutic effects would result from chemoembolization, ie, a combination of blood flow obstruction (with infarction) and prolonged, intensified drug exposure.

As previously mentioned, another approach using biodegradable starch microspheres has been recently developed (60-62). The concurrent hepatic-arterial injection of a suspension of starch microspheres in a drug solution has the potential of temporarily holding the drug solution in the hepatic-arterial capillary bed, thus allowing more time for the higher drug concentration to move into surrounding tissue. Carmustine and mitomycin have been examined in conjunction with starch microspheres given via the hepatic artery (62,70,71). These agents were chosen due to their rapid tissue uptake and mechanism of action as alkylating agents (ie, peak drug levels are important). Because of increased drug delivery to the liver and hepatic tumor, systemic drug exposure was reduced up to 90% for carmustine and 70% for mitomycin when the drug was given with starch microspheres versus drug injection alone. In hypervascular regions of tumors, more drug solution should be held up as compared to the drug entrapment in less vascular regions of

TABLE 4.—Colorectal cancer tumor nodules—hepatic tumor and liver microcirculatory vascularity determined by tomographic nuclide angiography

Lesion No.	Diameter (cm)*	Hypovascular core (cm)*	Tumor vascularity (cm)*	MAA activity: hypervascular area / Normal liver
1	2.4	None	Solid	4.6
2	3.0	None	Solid	3.4
3	3.6	None	Solid	2.7
4	4.2	None	Solid	2.5
5	4.8	None	Solid	2.6
6	5.4	None	Solid	5.4
7	6.6	None	Solid	6.4
8	8.4	None	Solid	3.4
9	9.6	2.4	Rim (3.6)	4.6
10	10.2	5.4	Rim (2.4)	4.5
11	11.4	5.4	Rim (3.0)	5.3
12	12.6	4.2	Rim (4.2)	2.5
13	15.6	8.4	Rim (3.6)	2.7

*Mean (variance of ± 20%).

normal liver. As the microspheres are digested, their diameter progressively decreases (from 40 μm initially) and the drug column moves distally into the capillary bed. Due to complete dissolution of the starch microspheres by 30 minutes postinjection, subsequent doses can be administered without destroying access to the tumor microcirculation. Repeated dosage studies and clinical trials comparing hepatic-arterial carmustine and mitomycin with and without starch microspheres are in progress.

Yttrium-90 resin microspheres with a 55-μm diameter have been used both in model systems (72) and in the clinic to treat hepatic tumors with internal radiotherapy (73,74). In the Walker carcinosarcoma rat liver tumor, yttrium-90 microspheres lodge in highest concentrations in the hypervascular tumor periphery (66). Concurrent epinephrine administration decreased yttrium-90 microsphere concentration in normal liver and markedly increased concentrations in the tumor in this rat model. In several treated patients with liver metastases, tumor nodules were found to have threefold to fourfold higher yttrium-90 microsphere levels than normal liver (74,75). Yttrium-90 microspheres have a short effective kill distance of 2-3 mm and deliver most of their dose over about 3 days. Tomographic nuclear angiography (*see* above) with TcMAA may be essential to the safe use of yttrium, as well as of starch microspheres, in that the therapeutic microspheres must go to the tumor for efficacy and not to the stomach, duodenum, or lungs if severe toxicity is to be prevented.

BUDR is an analog of thymidine, which is incorporated solely and specifically into the DNA of replicating cells. BUDR undergoes debromination in the liver and, in analogy with FUDR and thymidine (37,43), can be expected to have high hepatic extraction. BUDR has pharmacokinetic properties, including a high total-body clearance (5-15 L/minute), making it suitable for hepatic-arterial infusion with selective regional effects (76). BUDR administered by hepatic-arterial infusion should be incorporated selectively into proliferating tumor within the liver, since there is usually little hepatic parenchymal DNA synthesis. For maximum incorporation of BUDR into DNA, prolonged exposure is necessary (77-79). Such prolonged exposure should be possible using the totally implanted system for hepatic-arterial infusion described below. With sufficient incorporation into both strands of DNA, radiation sensitivity can be increased at least twofold. Studies with BUDR and yttrium-90 microspheres have not been done, but the combination would be expected to have considerable selective antitumor potential. Preclinical studies with this combination are in progress. The implanted system should allow continuous infusion of BUDR via the central pumping mechanism, whereas the pump sideport can be utilized for yttrium-90 microsphere injection. Further selectivity using both starch and yttrium microspheres may be possible through the concurrent administration of vasoconstrictors to shift flow from normal liver into the tumor microcirculation.

Dose Response

In addition to being pharmacokinetically rational, a second element in the evaluation of the appropriateness of a drug for intra-arterial chemotherapy is the dose-response curve for that given drug in the tumor type in question. By arterial infusion, one attempts to expose the tumor to more drug than is otherwise possible. However, if the tumor is extremely sensitive to a given drug, then the exposure with iv chemotherapy may be sufficient to kill the entire sensitive cell population. If, on the other hand, the tumor is absolutely resistant to the drug, then even increasing the exposure by 100-fold, as is possible with hepatic-arterial infusion of some drugs (table 3), may not produce noticeable antitumor response. If an agent is reported to have marginal activity when given iv in a particular tumor, then exploiting the dose-response curve through hepatic-arterial infusion may be profitable (2). For example, FU and FUDR both have definite, but marginal, activity in colorectal cancer. Hence, generating a marked increase in exposure by hepatic-arterial infusion of FU or FUDR is more likely to be of benefit than the use of another agent, such as cytarabine, which has essentially no activity in this disease (although the use of cytarabine would be appropriate pharmacokinetically).

When the dose response for an agent is sufficiently steep and the pharmacokinetics appropriate, it should be possible to generate cytotoxic drug levels in the hepatic-arterial tree with lower, nontoxic systemic levels. In fact, this should be reflected in the toxicity observed; ie, dose-limiting toxicity should be regional (liver), rather than systemic (bone marrow). It should be noted that the doses and schedules of agents can be selected to achieve maximum tolerated systemic levels with supramaximal effects on the tumor in the liver. For example, this is possible with FU despite its 50%-80% hepatic extraction, where a constant hepatic-arterial infusion of 1000 mg/m^2/day produces the same steady-state systemic level as 300 mg/m^2/day given iv (36,37). Three times the systemic dose can be given by the hepatic artery to achieve at least a 50-fold increased exposure to the liver tumor, yet the same systemic exposure (including extrahepatic tumor) occurs as is produced by iv infusion.

Schedule Dependency

A third consideration in successful intra-arterial chemotherapy is knowledge of the drug's schedule dependency. This is best defined in preclinical animal and cell culture experiments (80). Schedule dependency is based on the biochemical and cytokinetic actions of the agent in question. For example, in the case of alkylating agents, bolus injections for short-term peak exposure may produce maximal damage to the nucleic acids of cells. Lower drug levels with constant infusions can allow rate-limited repair processes to occur more efficiently and thereby prevent lethality (81). Alternatively, antimetabolites generally produce cytotoxicity as a function of duration of exposure above a given concentration (82). The drug schedule dictates the type of delivery system necessary. Short infusions or boluses may be given by hand injection or by a temporary pump attachment. Prolonged controlled infusions will require a pumping system. With improved drug delivery systems, there has been a recent surge in interest in protracted constant infusions (83). There is evidence that toxic effects are modified by such prolonged infusions, although evidence for improved efficacy requires controlled clinical trials.

Adequacy of Intra-arterial Infusion

Intra-arterial chemotherapy presents several unique difficulties not found with systemic, iv drug administration. Although a drug may be pharmacologically rational, it must be delivered directly into the tumor blood supply in a reliable and reproducible manner. For example, with protracted (ie, > 3 weeks) hepatic-arterial infusion using percutaneous angiographic catheters or surgically placed stiff plastic catheters, progressive hepatic-arterial thrombosis occurs at a high rate and gradually produces decreased blood flow and, ultimately, occlusion (52,84). Any improvement in response (53) (brought about through combined ischemia and higher drug levels generated) must be tempered by the inability to use further arterial chemotherapy. It also has been shown that the pattern of distribution of agents injected intra-arterially is dependent on the flow rate of the infusion (49). Radionuclide angiography using TcMAA appears to give the best representation of drug distribution at the low infusion rates (3-1000 ml/day) clinically used (50). When percutaneous angiographic catheters are used, the tip of the catheter tends to move about, leading to frequent and unpredictable flow distribution changes. In hepatic-arterial infusions when drug is infused inadvertently into a gastric artery, severe gastric damage can result (85,86). Hence, a catheter system free of thrombosis and movement which reliably infuses only the tumor-containing region or organ is a prerequisite for maximum response with minimal toxicity. The more potent the cytotoxic agent for intra-arterial use, the more important the focusing of the flow from the catheter. Surgical placement of soft silastic catheters in a manner to minimize turbulence in blood flow represents the most durable intra-arterial access at present (87). Nuclide angiography to guide in catheter placement and to ascertain complete drug flow distribution is equally important (fig 2) (88).

Drug Delivery Systems for Intra-arterial Use

Intra-arterial drug infusions have been limited by the type of pumping system involved. Pumps worn externally by patients have proven inconvenient because of their bulkiness and have been a major hindrance to normal daily activities. The pump and the external catheter connection have represented sites for the interruption of the infusion path leading to bleeding, catheter thrombosis, or infection from the introduction of pathogenic organisms. The need for a team of supportive personnel to maintain prolonged outpatient infusions and

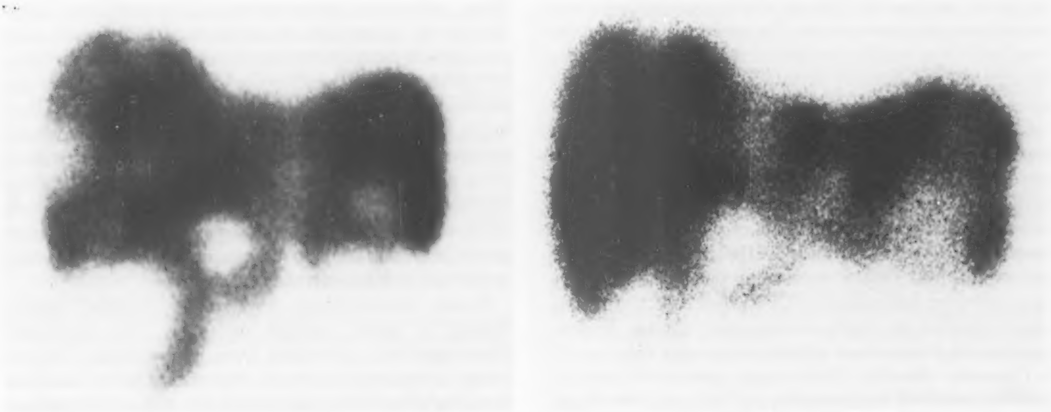


FIGURE 2.—Pre- and post-treatment liver scans in a patient receiving FUDR only to the right lobe via a right hepatic-arterial catheter. Post-treatment scan (right) shows regression of lesions in the right lobe, while untreated lesions in the left lobe have enlarged, demonstrating the need to infuse chemotherapy to the entire liver (2 catheters, 1 for right and 1 for left hepatic artery, are usually needed in such patients).

the clumsiness and unreliability of the infusion systems have limited their widespread applicability. For one or two short infusions of a week or less and for situations where agents of unknown activity are being evaluated by constant infusion, an external pump may have a role in some patients, despite the inconvenience and risk.

A totally implanted drug delivery system tested over the past several years appears to overcome many of the problems noted above and has been applied to intra-arterial infusional therapy of head and neck cancer (89,90), brain tumors (8), and liver cancer (4,5,91-95). The most extensive experience has been with hepatic-arterial chemotherapy where, at laparotomy, a small flexible silastic catheter is implanted to infuse the entire liver. Appropriate vessels are ligated in an effort to confine drug flow to the liver (87). The catheter is attached to a subcutaneously placed Model 400 Infusaid pump. In the first 93 patients with colorectal cancer metastatic to liver treated with this system at the University of Michigan, there were only two hepatic-arterial occlusions and one permanent catheter occlusion (91,95). Flow distribution to the entire liver ascertained by radionuclide angiography was maintained in the other 90 patients. Autopsies in six patients having catheters in place for up to 18 months showed no evidence of thrombosis or vessel wall damage in the catheterized artery. These patients, as a group, received 40,000 cumulative days of hepatic-arterial infusion and 3000 pump refills without a bleeding or infectious episode. On > 95% of the days, infusions were given on an outpatient basis. Patients are able to function relatively normally since the system is totally implanted under the skin. This system has been found to function reliably for other investigators as well (92-94). The Model 400 Infusaid pump has a sideport allowing direct injection into the hepatic-artery catheter. The 0.63-mm lumen on the hepatic-arterial catheter readily allows the passage of TcMAA, starch microspheres, and other forms of microspheres. The recent approval by the Food and Drug Administration of the Infusaid pump for hepatic-arterial infusion with FUDR has led to widespread use of the device.

Preliminary therapeutic results in the treatment of hepatic cancers have been excellent with the implanted system (91,95). Hepatic tumor response ascertained by physical examination and liver scan using standard criteria has been seen in about 80% of patients (91,93,95). Response durations to initial FUDR therapy averaged 8-9 months (95). Hepatic-arterial mitomycin through the pump sideport was added to FUDR when response was no longer evident or when extrahepatic disease became manifest; it produced an additional average response of 6-7 months' duration. Ninety-eight percent of patients with an elevated carcinoembryonic antigen level have had at least a 50% fall in levels with treatment. Median survival from diagnosis of liver metastases in patients with disease confined to the liver at pump-catheter implantation has been 25 months. For patients with disease outside of the liver at time of system implant, median survival was 12 months from time of diagnosis of liver metastases. Toxic effects were all reversible and consisted of mild gastritis (56% of patients), ulcers (8%), and anicteric (23%) and icteric (23%) hepatitis. With dose schedule modifications, all patients showing toxic effects were able to continue treatment without recurrence of toxicity.

Overview on Intra-arterial and Regional Chemotherapy

It is likely that much of the experience with hepatic-arterial therapy can be extrapolated to other body regions. For example, preliminary results in head and neck cancer suggest that the Infusaid pump with two silastic catheters, one placed in the external carotid artery and the other in the thyrocervical trunk, provides complete infusion to the regional tumor monitored by TcMAA nuclide flow scans (89,90,96). Such studies indicate that head and neck tumors are likewise hypervascular and may thus be susceptible to the microsphere and vasoconstrictor approaches described above for liver tumors. It is likely that similar methodology will be applicable to pelvic tumors and tumors of the extremities.

A major problem with regional chemotherapy that successfully controls regional tumor is extraregional tumor growth. For example, in our studies with hepatic-arterial therapy for colorectal liver metastases, > 80% of the patients died because of extrahepatic tumor progression; essentially no patients died of liver disease progression alone (95). This contrasts with a recent retrospective analysis of patients with colorectal liver metastases treated at the Dana-Farber Cancer Institute. In their study, 86% of the patients died of "liver failure" (97). Thus, effective hepatic-arterial chemotherapy may change the natural history of the disease. However, only randomized prospective studies using the best drug delivery technology and monitoring techniques will define the survival advantage achieved with this modality. Systemic failure with hepatic-arterial FUDR is not totally unexpected, since FUDR is 95% extracted by the liver when given by a hepatic-arterial infusion (37). Thus, drug regimens with potential systemic effect against occult (microscopic), extrahepatic disease will need to be integrated into such trials to determine whether it is possible to prevent or delay extrahepatic failure.

Recent developments have made regional chemotherapy a more rational endeavor. The important pharmacokinetic principles have been defined. The increase in regional exposure achieved is a direct function of a drug's total-body clearance and is an inverse function of the permeability-area product (defining ease of egress) for third spaces or of the regional arterial blood flow for intra-arterial infusion. Agents having appropriate properties are available. For new agents with appropriate pharmacokinetic parameters, regional chemotherapy may provide a means to examine the dose response against measurable tumors in the regions in question.

The observation that most tumors are hypervascular may be crucial to the development of selective treatments using microspheres to deliver therapy in direct proportion to the density of the microvasculature. Regionally infused vasoconstrictors (epinephrine and angiotensin) may allow shunting of flow away from normal tissue toward tumor without potentially serious systemic cardiovascular effects. Investigations and applications of regional chemotherapy have been fostered immensely by the reliability and convenience of the Infusaid implantable pump and by implantable injection ports. The problem of systemic failure with regional approaches still remains, but is approachable using pharmacologically rational programs aimed at delivering maximal systemic chemotherapy along with regional treatment. In hepatic-arterial therapy, in particular, a number of randomized prospective studies have recently been initiated to examine the response rate and survival with hepatic-arterial therapy versus systemic therapy versus combined systemic-hepatic-arterial treatment for metastatic colorectal cancer to liver. Other randomized studies are under way with ip therapy. Thus, the future for regional chemotherapy appears exciting. There are many opportunities to apply therapeutic principles rationally with the potential for significant benefit to many patients.

REFERENCES

1. LATHAM F, JR, and FOSTER JH. Hepatic resection for metastatic cancer. Am J Surg 113:551-557, 1967.
2. FREI, E, III, and CANELLOS GP. Dose: a critical factor in cancer chemotherapy. Am J Med 69:585-594, 1980.
3. BLACKSHEAR PJ, DORMAN FD, BLACKSHEAR PJ, JR, ET AL. A permanently implantable self-recycling low flow constant rate multipurpose infusion pump of simple design. Surg Forum 21:136, 1970.
4. BUCHWALD H, GRANGES TB, PERICLES P, ET AL. Intraarterial infusion chemotherapy for hepatic carcinoma using a totally implantable infusion pump. Cancer 45:866-869, 1980.
5. ENSMINGER W, NIEDERHUBER J, DAKHIL S, ET AL. Totally implanted drug delivery system for hepatic arterial chemotherapy. Cancer Treat Rep 65:393-400, 1981.
6. GREENBERG HS, TAREN J, ENSMINGER WD, ET AL. Benefit from and tolerance to continuous intrathecal infusion of morphine for intractable cancer pain. J Neurosurg 57:360-364, 1982.
7. DAKHIL S, ENSMINGER W, KINDT G, ET AL. Implanted system for intraventricular drug infusion in central nervous system tumors. Cancer Treat Rep 65:401-411, 1981.
8. PHILLIPS TW, CHANDLER WF, KINDT GW, ET AL. A new implantable continuous and bolus intra-carotid drug delivery system for the treatment of malignant gliomas. Neurosurgery 11:213-218, 1982.
9. ROHDE TD, BLACKSHEAR PJ, VARCO RL, ET AL. One year of heparin anticoagulation in an ambulatory subject using a totally implantable infusion pump. Minn Med 60:719-722, 1977.
10. BUCHWALD H, BARBOSA J, VARCO RL, ET AL. Treatment of a type II diabetic by a totally implantable insulin infusion device. Lancet 1:1233-1234, 1981.
11. SCHADE DS, EATON RP, EDWARDS WS, ET AL. A remotely programmable insulin delivery system—successful short-term implantation in man. JAMA 247:1846-1854, 1982.
12. GYVES JW, ENSMINGER WD, NIEDERHUBER J, ET AL. Totally implanted system for intravenous chemotherapy in patients with cancer. Am J Med 73:841-858, 1982.
13. SHAPIRO WR, YOUNG DF, and MEHTA BM. Methotrexate: distribution in cerebrospinal fluid after intravenous, ventricular and lumbar injections. N Engl J Med 293:161-166, 1975.
14. BLEYER WA, and POPLACK DG. Clinical studies of the central nervous system pharmacology of methotrexate. *In* Clinical Pharmacology of Anti-Neoplastic Drugs (Pinedo HM, ed). Elsevier/North Holland Biomedical Press, 1978, pp 115-131.
15. DEDRICK RL, MYERS CE, BUNGAY PM, ET AL. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep 62:1-11, 1978.
16. SPEYER JL, COLLINS JM, DEDRICK RL, ET AL. Phase I and pharmacological studies of 5-fluorouracil administered intraperitoneally. Cancer Res 40:567-572, 1980.
17. HOWELL SB, PFEIFLE CL, WUNG WE, ET AL. Intraperitoneal cisplatin with systemic thiosulfate protection. Ann Intern Med 97:845-851, 1982.
18. HOWELL SB, CHU BBF, WUNG WE, ET AL. Long-duration intracavitary infusion of methotrexate with systemic leucovorin protection in patients with malignant effusions. J Clin Invest 67:1161-1170, 1981.
19. BLEYER WA, and DEDRICK RL. Clinical pharmacology of intrathecal methotrexate. I. Pharmacokinetics in nontoxic patients after lumbar injection. Cancer Treat Rep 61:703-708, 1977.
20. DAKHIL S, ENSMINGER WD, STROTHER V, ET AL. Pharmacokinetics of intraventricular 5-fluoro-2′-deoxyuridine (FUDR) in patients with meningeal neoplasia. Proc Am Assoc Cancer Res and ASCO 22:178, 1981.
21. RIESELBACH RE, CHIRO GD, FREIREICH EJ, ET AL. Subarachnoid distribution of drugs after lumbar injection. N Engl J Med 267: 1273-1278, 1962.
22. BLEYER WA, POPLACK DG, SIMON RM, ET AL. "Concentration × time" methotrexate via a subcutaneous reservoir: a less toxic regimen for intraventricular chemotherapy of central nervous system neoplasms. Blood 50:835-842, 1978.
23. PINEDO HM, and CHABNER BA. Role of drug concentration, duration of exposure, and endogenous metabolites in determining methotrexate cytotoxicity. Cancer Treat Rep 61:709-715, 1977.
24. GUTIN PH, WEISS HD, WIERNIK PH, ET AL. Intrathecal *N*, *N′*, *N′′*-triethyienethiophosphoramide [thio-tepa (NSC 6396)] in the treatment of malignant meningeal disease. Cancer 38:1471-1475, 1976.
25. GYVES J, ENSMINGER W, NIEDERHUBER J, ET AL. Phase I study of intraperitoneal (ip) 5 day continuous 5-FU infusion and bolus mitomycin C. Proc ASCO 1:C-59, 1982.
26. SPEYER JL, SUGARBAKER PH, COLLINS JM, ET AL. Portal levels and hepatic clearance of 5-fluorouracil after intraperitoneal administration in humans. Cancer Res 41:1916-1922, 1981.
27. OZOLS RF, YOUNG RC, SPEYER JL, ET AL. Phase I and pharmacological studies of adriamycin administered intraperitoneally to patients with ovarian cancer. Cancer Res 42:4265-4269, 1982.
28. JONES RB, COLLINS JM, MYERS CE, ET AL. High-volume intraperitoneal chemotherapy with methotrexate in patients with cancer. Cancer Res 41:55-59, 1981.
29. CHABNER BA, STOLLER RG, HONDE K, ET AL. Methotrexate disposition in humans: case studies in ovarian cancer and following high dose infusion. Drug Metab Rev 8:107-117, 1978.
30. ACKERMAN NB, LIEN WM, KONDI ES, ET AL. The blood supply of experimental liver metastases. I. The distribution of hepatic artery and portal vein blood to "small" and "large" tumors. Surgery 66:1067-1072, 1969.
31. BREEDIS C, and YOUNG G. Blood supply of neoplasms in the liver. Am J Pathol 30:969-985, 1954.
32. TAYLOR I, ROWLING J, and WEST C. Adjuvant cytotoxic liver perfusion for colorectal cancer. Br J Surg 66:823-837, 1979.
33. ECKMAN WW, PATLAK CS, and FENSTERMACHER JD. A critical evaluation of principles governing the advantages of intraarterial in-

fusions. J Pharmacokinet Biopharm 2:257-285, 1974.

34. CHEN H-SG, and GROSS JF. Intra-arterial infusion of anticancer drugs: theoretic aspects of drug delivery and review of responses. Cancer Treat Rep 64:31-40, 1980.
35. COLLINS JM, and DEDRICK RL. Pharmacokinetics of anticancer drugs. *In* Pharmacologic Principles of Cancer Treatment (Chabner B, ed). W. B. Saunders Co, 1982, pp 77-99.
36. ENSMINGER W, STETSON P, GYVES J, ET AL. Dependence of hepatic arterial fluorouracil pharmacokinetics on dose rate and duration of infusion. Proc ASCO 2:C-98, 1983.
37. ENSMINGER WD, ROSOWSKY A, RASO V, ET AL. A clinical pharmacologic evaluation of hepatic arterial infusions of 5-fluoro-2′-deoxyuridine and 5-fluorouracil. Cancer Res 38:3784-3792, 1978.
38. ENSMINGER WD, THOMPSON M, COME S, ET AL. Hepatic arterial BCNU: a pilot clinical-pharmacologic study in patients with liver tumors. Cancer Treat Rep 62:1509-1512, 1978.
39. GYVES J, ENSMINGER W, STETSON P, ET AL. Clinical pharmacology of mitomycin C (mito) by hepatic arterial (HA) infusion. Proc ASCO 2:C-97, 1983.
40. KELSEN DP, HOFFMAN J, ALCOCK N, ET AL. Pharmacokinetics of regional infusion of cisplatin. Proc Am Assoc Cancer Res and ASCO 21:745, 1980.
41. ENSMINGER WD, DAKHIL S, DOAN K, ET AL. Clinical pharmacology of dichloromethotrexate in hepatic arterial infusions. Proc Am Assoc Cancer Res and ASCO 22:271, 1981.
42. TSENG MH, LUCH J, MITTELMAN A, ET AL. Chemotherapy of advanced colorectal cancer with regional arterial mitomycin C infusion and concomitant measurement of serum drug level. Proc Am Assoc Cancer Res and ASCO 22:359, 1981.
43. ENSMINGER WD, and FREI E, III. High-dose intravenous and hepatic artery infusions of thymidine. Clin Pharmacol Ther 24:610-615, 1978.
44. SONNENFELD P, SCHWADE JE, RICCARDI R, ET AL. Clinical pharmacology of bromodeoxyuridine (NSC 38297): a nonhypoxic radiosensitizer. Proc Am Assoc Cancer Res 23:488, 1982.
45. COLLINS JM, DEDRICK RL, KING FG, ET AL. Nonlinear pharmacokinetic models for 5-fluorouracil in man: intravenous and intraperitoneal routes. Clin Pharmacol Ther 28:235-246, 1980.
46. KIRKWOOD J, ENSMINGER W, and ROSOWSKY A. 5-Fluorouracil and thymidine: pharmacokinetic observations in a phase I trial comparing 5-fluorouracil and 5-fluorouracil with concurrent thymidine infusions. Cancer Res 40:107-113, 1980.
47. RAMMING KP, SPARKS FC, EILBER FR, ET AL. Hepatic artery ligation and 5-fluorouracil infusion for metastatic colon carcinoma and primary hepatoma. Am J Surg 132:236-242, 1976.
48. KARAKOUSIS CP, DOUGLASS HO, JR, and HOLYOKE ED. Technique of infusion chemotherapy, ligation of the hepatic artery and dearterialization in malignant lesions of the liver. Surg Gynecol Obstet 149:403-407, 1979.
49. KAPLAN WD, D'ORSI CJ, ENSMINGER WD, ET AL. Intra-arterial radionuclide infusion: a new technique to assess chemotherapy perfusion patterns. Cancer Treat Rep 62:699-703, 1978.
50. KAPLAN WD, ENSMINGER WD, COME SE, ET AL. Radionuclide angiography to predict patient response to hepatic artery chemotherapy. Cancer Treat Rep 64:1217-1222, 1980.
51. DAHL EP, FREDLUND PE, TYLEN U, ET AL. Transient hepatic dearterialization followed by regional intra-arterial 5-fluorouracil infusion as treatment for liver tumors. Ann Surg 193:82-88, 1981.
52. CLOUSE ME, AHMED R, RYAN RB, ET AL. Complications of long term transbrachial hepatic arterial infusion chemotherapy. AJR 129: 799-803, 1977.
53. PATT YZ, WALLACE S, FREIREICH EJ, ET AL. The palliative role of hepatic arterial infusion and arterial occlusion in colorectal carcinoma metastatic to the liver. Lancet 1:349-350, 1981.
54. BENGMARK S, and ROSENGREN K. Angiographic study of the collateral circulation to the liver after ligation of the hepatic artery in man. Am J Surg 19:620-624, 1970.
55. WATKINS E, JR, HERING AC, LUNA R, ET AL. The use of intravascular balloon catheters for isolation of the pelvic vascular bed during pump-oxygenator perfusion of cancer chemotherapeutic agents. Surg Gynecol Obstet 111:464-468, 1960.
56. CHO KJ, ENSMINGER WD, SHIELDS JJ, ET AL. Selective tissue ablation by pharmacoangiography. *In* Angiography (Abrams HL, ed). Boston, Little, Brown and Co, 1983, pp 2175-2190.
57. ABRAMS HL. The response of neoplastic renal vessels to epinephrine in man. Radiology 82:217-224, 1964.
58. HAFSTROM L, NOBIN A, PERSSON B, ET AL. Effects of catecholamines on cardiovascular response and blood flow distribution to normal tissue and liver tumor in rats. Cancer Res 40:481-485, 1980.
59. IWAKI A, NAGASUE N, KOBAYOSHI M, ET AL. Intra-arterial chemotherapy with concomitant use of vasoconstrictors for liver cancer. Cancer Treat Rep 62:145-146, 1978.
60. ARONSEN KF, HELLEKANT C, HOLMBERG J, ET AL. Controlled blocking of hepatic artery flow with enzymatically degradable microspheres combined with oncolytic drugs. Eur Surg Res 11:99-106, 1979.
61. LINDELL B, ARONSEN KF, NOSSLIN B, ET AL. Studies in pharmacokinetics and tolerance of substance temporarily retained in the liver by microspheres embolisation. Ann Surg 187:95-99, 1978.
62. DAKHIL S, ENSMINGER W, CHO K, ET AL. Improved regional selectivity of hepatic arterial BCNU with degradable microspheres. Cancer 50:631-635, 1982.
63. WARREN BA. The vascular morphology of tumors. *In* Tumor Blood Circulation: Angiogenesis, Vascular Morphology and Blood Flow of Experimental and Human Tumors (Peterson HI, ed). Florida, CRC Press, Inc, 1979, pp 1-47.
64. GYVES J, ENSMINGER W, YANG P, ET AL. Clinical utility of microspheres to assess and attack hepatic tumor microcirculation. Clin Res 30:418A, 1982.
65. GYVES J, ENSMINGER W, THRALL J, ET AL. Dependence of hepatic tumor vascularity on tumor size. Clin Res 30:747A, 1982.
66. ACKERMAN NB, and HECKMER PA. The blood supply of experimental liver metastases. V. Increased tumor perfusion with epinephrine. Am J Surg 140:625-631, 1980.
67. MATTSSON J, APPELGREN L, HAMBERGER B, ET AL. Tumor vessel innervation and influence of vasoactive drugs on tumor blood flow. *In* Tumor Blood Circulation: Angiogenesis, Vascular Morphology and Blood Flow of Experimental and Human Tumors (Peterson HI, ed). Florida, CRC Press, Inc, 1978, pp 129-141.
68. KATO T, NEMOTO R, MORI H, ET AL. Sustained-release properties of microencapsulated mitomycin C with ethylcellulose infused into the renal artery of the dog kidney. Cancer 46:14-21, 1980.
69. ______. Arterial chemoembolization with mitomycin C microcapsules in the treatment of primary or secondary carcinoma of the kidney, liver, bone, and intrapelvic organs. Cancer 48:674-680, 1981.
70. GYVES J, ENSMINGER W, VANHARKEN D, ET AL. Improved regional selectivity of hepatic arterial mitomycin C by starch microspheres. Proc Am Assoc Cancer Res 23:137, 1982.
71. ______. Improved regional selectivity of hepatic arterial mitomycin by starch microspheres. Clin Pharmacol Ther 34:259-265, 1983.
72. BLANCHARD RJW, GROTENHUIS I, LAFARE JW, ET AL. Blood supply to hepatic V2 carcinoma implants as measured by radioactive microspheres. Proc Soc Exp Biol Med 118:465-468, 1965.
73. ARIEL IM. Treatment of inoperable primary pancreatic and liver cancer by the intra-arterial administration of radioactive isotopes (Y^{90} radiating microspheres). Ann Surg 162:267-278, 1965.
74. GRADY E. Internal radiation therapy of hepatic cancer. Dis Colon Rectum 22:371-375, 1979.
75. GRADY ED, AUDA SP, and CHEEK WV. Vasoconstrictors to improve localization of radioactive microspheres to treat liver cancer. *In* Proceedings of the 1980 Medical Association of Georgia Scientific Assembly (Georgia Chapter, American College of Surgeons), Atlanta GA, Nov 21, 1980.
76. SCHWADE JG, MYERS CM, and SONNENFELD P. Bromodeoxyuridine (NSC 38297) pharmacokinetics and clinical results with intravenous administration. Radiat Oncol Biol Phys 7:9, 1981.
77. DJORDJEVIC B, and SZYBALSKI W. Genetics of human cell lines. III. Incorporation of 5-bromo- and 5-iododeoxyuridine into the deoxy-

ribonucleic acid of human cells and its effects on radiation sensitivity. J Exp Med 112:509-531, 1960.

78. SANO K, HOSHINO T, and NAGAI M. Radiosensitization of brain tumor cells with a thymidine analogue (bromouridine). J Neurosurg 28:530-538, 1968.
79. SCHINDLER R, RAMSEIER L, and GRIEDER A. Increased sensitivity of mammalian cell cultures to radiomimetic alkylating agents following incorporation of 5-bromodeoxyuridine into cellular DNA. Biochem Pharmacol 15:2013-2023, 1966.
80. BLUM RH, FREI E, III, and HOLLAND JF. Principles of dose, schedule, and combination chemotherapy. *In* Cancer Medicine (Holland JF, and Frei E, III, eds). Philadelphia, Lea and Febiger, 1982, pp 730-752.
81. SKIPPER HE, and SCHABEL FM. Quantitative and cytokinetic studies in experimental tumor systems. *In* Cancer Medicine (Holland JF, and Frei E, III, eds). Philadelphia, Lea and Febiger, 1982, pp 663-685.
82. CHABNER BA, and YOUNG RC. Threshold methotrexate concentration for in vivo inhibition of DNA synthesis in normal and tumorous target tissues. J Clin Invest 52:1804-1811, 1973.
83. LOKICH J, BOTHE A, FINE N, ET AL. The delivery of cancer chemotherapy by constant venous infusion. Cancer 50:2731-2735, 1982.
84. CADY B. Hepatic arterial patency and complications after catheterization for infusion chemotherapy. Ann Surg 178:156-158, 1973.
85. CHUANG VP, WALLACE S, STROEHLEIN J, ET AL. Hepatic artery infusion chemotherapy: gastro-duodenal complication. AJR 137:347-350, 1981.
86. NARSETE T, ANSFIELD F, WIRTANEN G, ET AL. Gastric ulceration in patients receiving intrahepatic infusion of 5-fluorouracil. Ann Surg 186:734-736, 1977.
87. NIEDERHUBER JE, and ENSMINGER WD. Surgical considerations in the management of hepatic neoplasia. Semin Oncol 10:135-147, 1983.
88. YANG PJ, THRALL JH, ENSMINGER WD, ET AL. Perfusion scintigraphy (Tc-99m MAA) during surgery for placement of chemotherapy catheter in hepatic artery: concise communication. J Nucl Med 23:1066-1069, 1982.
89. BAKER SR, WHEELER RH, ENSMINGER WD, ET AL. Intraarterial infusion chemotherapy for head and neck cancer using a totally implantable infusion pump: a preliminary report. Head Neck Surg 4:118-124, 1981.
90. BAKER SR, and WHEELER RH. Long-term intra-arterial chemotherapy infusion of ambulatory head and neck cancer patients. J Surg Oncol 211:125-131, 1982.
91. ENSMINGER W, NIEDERHUBER J, GYVES J, ET AL. Effective control of liver metastases from colon cancer with an implanted system for hepatic arterial chemotherapy. Proc Am Assoc Cancer Res 1:94, 1982.
92. BARONE RM, BYFIELD JE, GOLDFARB PB, ET AL. Intraarterial chemotherapy using an implantable infusion pump and liver irradiation for the treatment of hepatic metastases. Cancer 50:850-862, 1982.
93. BALCH CM, URIST MM, and MCGREGOR ML. Continuous regional chemotherapy for metastatic colorectal cancer using a totally implantable infusion pump. Am J Surg 145:285-290, 1983.
94. COHEN AM, GREENFIELD A, and WOOD WC. Treatment of hepatic metastases by transaxillary hepatic artery chemotherapy using an implanted drug pump. Cancer 51:2013-2019, 1983.
95. NIEDERHUBER J, ENSMINGER WD, GYVES JW, ET AL. Regional chemotherapy of colorectal cancer metastatic to the liver. Cancer. In press.
96. BAKER SR, and WHEELER R. Intra-arterial chemotherapy of head and neck cancer. *In* Head and Neck Cancer: Advances in Treatment and Research (Wolf GT, and Chietien PB, eds). Boston, Martinus-Nijhoff. In press.
97. GOSLIN R, STEELE G, ZAMCHECK N, ET AL. Factors influencing survival in patients with hepatic metastases from adenocarcinoma of the colon or rectum. Dis Colon Rectum 25:749-754, 1982.

Human Tumor Colony Assay and Chemosensitivity Testing[1]

Sydney E. Salmon[2,3,*]

The human tumor colony assay (HTCA; clonogenic or tumor stem cell assay) is an in vitro culture system employing semisolid medium support. Using HTCA, the growth and chemosensitivity of clonogenic tumor cells present in fresh biopsy specimens of human tumors can be investigated. Since this technique was described, there has been a marked increase in the direct study of human tumors in vitro. Excellent evidence has been obtained which establishes that colonies grown in HTCA are comprised of tumor cells and that clonogenic cells within tumor colonies have the property of self-renewal (the defining property of a tumor stem cell). Chemosensitivity testing with specific agents in HTCA has documented striking degrees of heterogeneity in drug sensitivity from patient to patient, even for tumors of the same histopathology. Clinical correlations have been made between in vitro chemosensitivity and the response of patients with metastatic cancer to chemotherapy. In a series of trials, HTCA has had a 71% true-positive rate and a 91% true-negative rate for predicting the drug sensitivity and resistance, respectively, of cancer patients to specific chemotherapeutic agents. The assay thus appears to be a prognostic factor which identifies chemosensitive patients and which in the future may allow some individualization of chemotherapy. HTCA has also had several areas of application to new drug development and screening. While results obtained have been encouraging, ongoing developmental research is needed to improve growth rates for many tumor types and to further improve, standardize, and simplify assay methodology. With such improvements, it appears likely that there will be a marked increase in the use of such in vitro human tumor assays for chemosensitivity testing and drug development. [Cancer Treat Rep 68:117–125, 1984]

While there has been substantial progress in the development of anticancer drugs, a number of common cancers appear resistant to the available drugs. A major objective in cancer research has been to develop predictive tests for human cancers, which could be used to measure drug sensitivity and resistance both for clinical application and to enhance preclinical drug screening and development. Studies carried out in murine tumor models at the Ontario Cancer Institute had suggested that in vitro tissue culture assays performed in semisolid media (such as agar) could be used to assess chemosensitivity of clonogenic tumor cells (which are considered to be closely related to tumor stem cells in vivo) (1). Tumor stem cells are a key subpopulation of cells within a tumor; they are responsible for self-renewal of the tumor cell population, as well as recurrence and metastasis after subcurative local or systemic treatment (2,3). In in vitro tissue culture assays, murine myeloma stem cells appeared to have greater sensitivity to melphalan than did normal bone marrow progenitors. Such in vitro assays of survival of tumor colony-forming units (TCFU) after drug exposure proved to be predictive of response and survival in mouse myeloma (4). In the mid-1970s, Dr. Hamburger and I (5,6) developed a similar assay system which proved capable of supporting tumor colony formation from bone marrow aspirates from patients with multiple myeloma and tumor biopsy specimens from ovarian cancer and a variety of other neoplasms. A related assay procedure applied to trans-

---

[1] Supported by Public Health Service grants CA-21839, CA-17094, and CA-23074 and contract N01-CM-17497 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[2] University of Arizona Cancer Center, Tucson.

[3] I wish to thank Drs. David S. Alberts, Brian G. M. Durie, Frank Meyskens, and Thomas Moon for their participation in studies at the University of Arizona Cancer Center; Laurie Young, Joyce Lebowitz, Barbara Soehnlen, and Rosa Liu for skilled technical assistance; and Toni Meinke for preparation of this manuscript.

[*] *Reprint requests to:* Sydney E. Salmon, MD, University of Arizona Cancer Center, University of Arizona College of Medicine, Section of Hematology and Oncology, Arizona Health Sciences Center, Tucson, AZ 85724.

plantable human tumor xenografts provided a model preclinical system which suggested that in vitro chemosensitivity of human tumors could predict the response of xenografts of the same tumor in vivo in the mouse (7). A major objective has been to develop a reproducible, pharmacologically based colony assay system which would prove useful for studies of cancer biology, prediction of response and survival in cancer patients, and screening and assessment of new anticancer drugs. This paper briefly reviews progress in these areas, focusing particularly on the predictive chemosensitivity technique developed in our laboratories and applications of the human tumor colony assays (HTCA). Drug sensitivity testing studies with HTCA have provided substantial evidence for patient-to-patient heterogeneity in sensitivity of tumor cells to individual anticancer drugs (even among tumors of the same histopathology). Evidence has also been obtained indicating that the assay has prognostic value with respect to prospectively defining inherent sensitivity of clonogenic human tumor cells to selected standard and new anticancer drugs.

METHODS

Methodologic procedures described briefly below are those developed and carried out at the University of Arizona Cancer Center; with some variation, they have been adopted by most other laboratories and are, of course, subject to future improvement. The soft agar procedure of Hamburger and Salmon (5,6) was utilized for studies of multiple myeloma and the various solid tumors tested. Solid tumor biopsy specimens were disaggregated into single-cell suspensions with mechanical (8) or enzymatic (9,10) methods. Drug exposure was either for 1 hour prior to plating or continuous by incorporating the agent into the agar (11,12). The exposure mode was selected to simulate the likely clinical drug dosing or disposition for the specific drug tested. The anticancer drugs tested were obtained from the National Cancer Institute or the pharmaceutical industry. Whenever available, the clinical formulation of the drug was utilized, reconstituted in the appropriate diluents, and further diluted into complete tissue culture medium. Stable drugs were stored in replicate single-use tubes at suitable dilutions at -80°C. Aliquots of cells were exposed at 37°C to at least two concentrations of the drug so that a one-log dose-response curve could be constructed. Drugs were studied at low concentrations (each in triplicate) generally ranging up to 1.0 μg/ml. Procedures for cell plating have been detailed previously (6,8,13,14). Spleen-conditioned medium (5) was used only for the myeloma cultures.

All plates were examined by inverted microscopy on the day of plating. Our current positive control is with the plant lectin, abrin, added at 10 μg/ml by continuous exposure (15). These proliferation controls were counted simultaneously with the normally incubated control plates. Plates were scanned every few days by inverted microscopy and colonies were counted on Days 10-16 using a Bausch and Lomb FAS II image-analysis system which had been optimized for counting tumor colonies and excluding artifacts (16).

Currently, we accept colonies on the FAS II to be those proliferative cellular units of relatively circular shape and density, with a diameter of at least 60 μm. Use of an initial Day-1 FAS II count for background subtraction and a stain (iodophenyl-nitrophenyl-phenyltetrazolium) for selective counting of viable colonies further enhances the specificity of the assay (17,18). In our laboratory, the definition of adequate in vitro growth for evaluation of drug effect is a minimum of 30 colonies/35-mm Petri dish in the controls. Tumor colony formation is linear with the number of tumor cells plated. The median number of tumor colonies from a large series of countable experiments is 108 colonies/control dish (500,000 nucleated cells plated). Thus, the cloning efficiencies in this system are low but provide sufficient growth for drug evaluation in 40%-70% of tumors cultivated from various tumor types. Criteria for defining in vitro sensitivity for specific drugs were based on calculation of the percentage survival of drug-exposed plates relative to the control at "boundary" concentrations, which could be calculated to be readily achievable (generally 10% of the plasma concentration-time product). In the initial studies, the in vitro survival-concentration curves were ranked for sensitivity using an "area-under-the-curve technique" (13,19). More recently, a mathematically simpler ranking of the percent survival data up to empirically derived "cutoff" drug concentrations has been used (20). In each instance, correlations are drawn between the in vitro and in vivo data to form a "training set" (for drugs as well as tumor types) for prospective applications thereafter (19,20). At present, we classify tumors as sensitive to specific drugs if survival of TCFU is reduced to $\leqslant$ 30% of control, and as intermediate if survival is reduced to 30%-50% of control. When survival of TCFU is $>$ 50% of control at the cutoff concentration, the tumor is considered to be resistant to the specific drug tested. The first report on clinical correlations in myeloma and ovarian cancer represented an initial training set for a number of standard agents (13). Subsequent prospective clinical trials have been of either of two types: (*a*) prospective correlative trials or (*b*) prospective decision-aiding trials (21). In the prospective correlative trial, the laboratory tests the specific drug or drugs the patient is scheduled to receive after the biopsy is performed, and treatment is thus independent of the laboratory result and is carried out without knowledge of it. In the prospective decision-aiding trial, the patient's specific treatment is predicated on evidence of in vitro sensitivity to a specific drug or drugs. In all instances, the laboratory results are obtained by the laboratory's technical staff without influence from the physicians responsible for patient treatment and for independently assessing the patient's clinical response. Criteria for clinical response which were utilized were similar to those of the Southwest Oncology Group. Clinical drug sensitivity was defined as the achievement of at least a partial remission for all tumor types except melanoma, for which mixed responses were also considered to reflect drug effect. Stable disease was defined as treatment failure. Clinical correlations are being gathered (13,22-29) and effects of new agents in this assay have been reported (30-35) for a variety of tumor types.

RESULTS AND DISCUSSION

Growth Characteristics

A wide variety of tumors have successfully been grown in HTCA. As of 1980, most major forms of human cancer had been grown (36). Some of the recognized limitations of the HTCA in its current stage of development include difficulties in obtaining true single-cell suspensions and very low cloning efficiencies observed for many tumor types (eg, 0.01%-0.1%). While most tumor types can be grown in HTCA, the cloning efficiencies vary widely, and neoplasms such as lymphoma, multiple myeloma, and osteosarcoma have relatively poor growth rates. The best growth rates have been observed in ovarian cancer, with about 80% of tumor specimens giving rise to at least 30 colonies/plate. Growth in melanoma and lung cancer is also quite good. Overall, approximately 50% of specimens for various types of cancers give rise to at least 30 colonies/plate. A linear relationship is generally observed between the number of cells plated and the number of tumor colonies formed, up to the range of 5×10^5 to 1×10^6 cells/plate, after which the curve plateaus. Cell concentrations yielding linearity provide the basis for quantitation of drug effects in HTCA. Morphologic, cytogenetic, and biomarker studies have proven that the colonies are comprised of tumor cells (17). Colony formation by normal fibroblasts is inhibited by the agar in the culture system, whereas growth of normal hematopoietic precursors is limited by the lack of conditioning factors required for their growth. Tumor colonies arising from single tumor cells are by definition arising from clonogenic tumor cells, which are considered to be closely related to tumor stem cells in vivo. Evidence that tumor colonies contain cells with stem cell characteristics has been obtained by growth of tumors in nude mice from tumor colonies (eg, in lung cancer) (37) and by "self-renewal" studies in vitro. In vitro self-renewal studies involve disaggregation and replating of single cells from primary tumor colonies and observing secondary tumor colony formation. This has been achieved in melanoma (38) and ovarian cancer (39). Thus, it is apparent that at least some of the cells giving rise to colonies in HTCA are closely related to tumor stem cells. The low cloning observed may in fact reflect tumor stem cell characteristics but also could reflect nutritional deficiencies in the culture system (40).

In Vitro Sensitivity of TCFU

After a 1-hour exposure, survival curves for tumors exposed to cycle-nonspecific drugs generally show progressively increasing lethality with increasing drug dosage. However, substantial heterogeneity in sensitivity is observed from patient to patient. An example of this phenomenon is shown in figure 1 (24). The use of 1 hour as a routine incubation time for most drugs may be viewed as arbitrary (41) but was selected to encompass the major pharmacologic exposure time in vivo after iv push injection of typical short-acting anticancer drugs (12). Ongoing research to study effects of varying exposure times is of course warranted. Another important characteristic which should be manifest by cycle-nonspecific drugs is dose-dependent reduction in survival. Many of the apparently flat survival curves observed in early experiments appear to have been artifactual and were likely due to the presence of aggregates in the cell suspensions. Use of Day-1 subtraction counts, the positive control, and a viability stain has substantially reduced such problems. Drugs which are schedule-dependent often manifest flat survival concentration curves with a 1-hour exposure but steep inhibition curves at substantially lower doses when the drug is added to the agar for continuous exposure studies. An example of this phenomenon with bleomycin in ovarian cancer is illustrated in figure 2 (12). Schedule-dependent curves have been observed with bleomycin, etoposide, and vinblastine, but not with doxorubicin, cisplatin, or alkylating agents (42). With cycle-nonspecific agents, greater sensitivity is observed with continuous exposure as compared to 1 hour, but the difference is generally less than tenfold. In contrast, with schedule-dependent drugs, the degree of inhibition may be > 100-fold that observed with a 1-hour exposure. A common phenomenon observed with many of the standard anticancer drugs is that most solid tumor specimens exhibit resistance to the agent up to dose levels which exceed pharmacologically achievable plasma concentrations. For example, colorectal carcinoma specimens exhibit in vitro sensitivity to 5-FU only in about 25% of cases and less often to alkylating agents or vinblastine, whereas sensitivity is more often observed with these drugs when tested against ovarian cancer. Since marked sensitivity to achievable concentrations of standard agents is relatively infrequent, it is clear that relatively large tumor specimens are preferable to increase the probability that sensitivity will be observed to one or more agents. As shown in table 1, we have seen precisely this phenomenon in myeloma, melanoma, and ovarian cancer (20). Therefore, whenever possible we attempt to test at least seven or eight drugs (at two concentrations) to increase the probability of identifying at least one active drug. In ovarian cancer, the frequency of in vitro sensitivity to common agents such as cisplatin, doxorubicin, and bleomycin was greater in patients who had received no prior chemother-

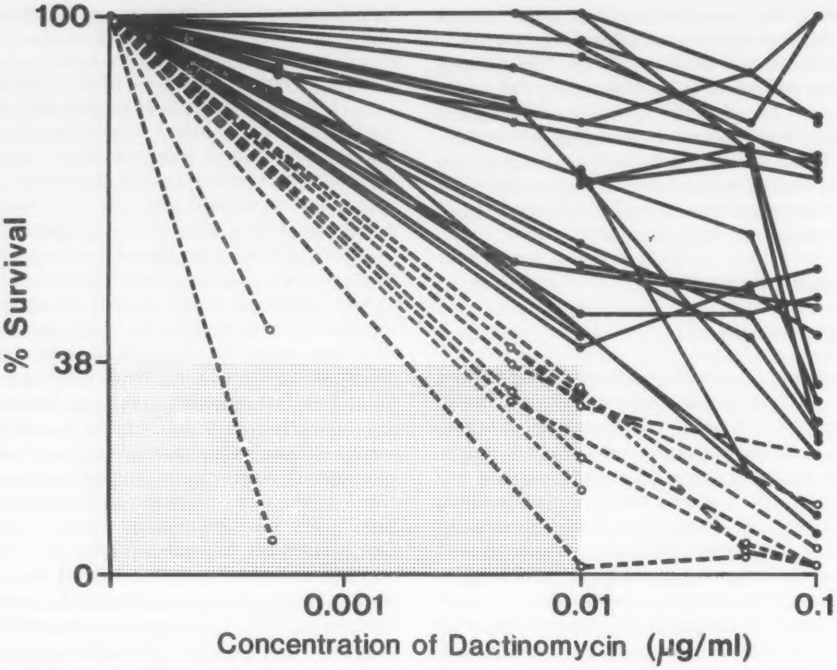


FIGURE 1.—Chemosensitivity of melanoma colony-forming units to dactinomycin. In this study the shaded zone was considered to reflect the relevant range of in vitro survival and drug concentrations to reflect in vitro sensitivity. Each line represents a different tumor biopsy tested. Lines that pass through the shaded zone of sensitivity are dashed (24).

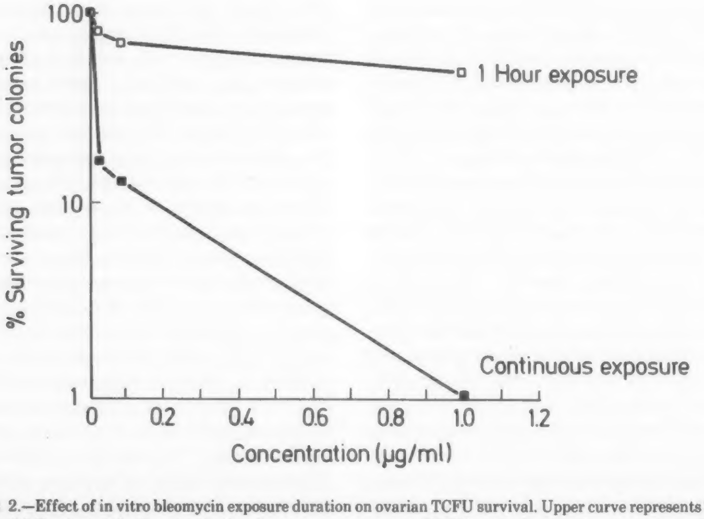


FIGURE 2.—Effect of in vitro bleomycin exposure duration on ovarian TCFU survival. Upper curve represents TCFU survival following a 1-hr drug incubation. Lower curve represents TCFU survival on continuous contact with the drug in the agar gel matrix (12).

TABLE 1.—Identification of drugs showing patient sensitivity in vitro, related to the No. of drugs tested*

No. of drugs tested in vitro	No. of patients sensitive† (%)	No. of patients resistant‡	Total No. of patients tested
≤ 3	13 (42)	18	31
4-7	82 (77)	24	106
7+	19 (100)	0	19
Total	114 (73)	42	156

*Reprinted from ref 20 with permission of the publisher.

†Sensitive in vitro to at least 1 of the drugs tested.

‡Resistant to all drugs tested in vitro.

apy than in those who were in relapse (23). However, this phenomenon was not observed in melanoma (24), a tumor type for which it is more difficult to define effective drugs (even for previously untreated patients).

Serial in vitro studies have also been evaluated in a series of 12 patients who underwent 45 sequential tumor biopsies before and at various stages after initiation of therapy (11). Table 2 summarizes this experience. Overall, we observed that tumors which exhibited sensitivity to a specific drug in vitro prior to therapy frequently showed resistance in vitro on re-biopsy after the patient had relapsed (at least 50% increase in the area under the survival-concentration curve) after treatment with that agent; none showed increased sensitivity. In contrast, patients who received no therapy or unrelated therapy did not have a significant increase in the frequency of resistance in the follow-up in vitro tests ($P = 0.03$) (14).

In Vitro Drug Testing and Correlation With Clinical Response

Correlation between in vitro chemosensitivity in HTCA and clinical response was first established at the University of Arizona Cancer Center (13). While the initial patient population reported was small (nine patients with myeloma and nine with ovarian cancer), a total of 31 clinical trials could be analyzed for this patient group. A highly significant correlation was observed between in vitro tumor resistance to specific drugs and failure of the patient to respond to the same drugs clinically. On the other hand, in vitro sensitivity was also noted in most instances where the patient responded to the drug; however, there was a subset of patients whose TCFU exhibited in vitro sensitivity but failed to show objective clinical response (false positives) (13). While this initial report clearly identified the potential of HTCA for chemosensitivity testing, it also detailed a number of practical and logistic problems and scientific issues that would need resolution before broad clinical application of HTCA could become a reality. A number of subsequent studies have independently confirmed and extended our initial report. These have included both retrospective analyses (22-24,26,28,29,43) and, more recently, prospective correlative designs (12,25,27,44). The correlations between in vitro sensitivity and clinical response in trials from a series of centers are summarized in table 3. While there were some differences in specific criteria for in vitro sensitivity and methodology in these institutions, and some variation in sensitivity or response by tumor type, the composite overview is of considerable interest. With respect to prediction of objective response, overall, HTCA had a 71% true-positive rate and a 91% true-negative rate. Some differences have emerged in the results from retrospective and prospective trials. In one large prospec-

TABLE 2.—Correlations of 45 serial in vitro studies in 12 patients*

Clinical information	No. of biopsies	Area under the survival-concentration curve		
		Decrease (> 50%)	No change (< 50%)	Increase (> 50%)
No therapy given or unrelated therapy	25	0	22	3
Specific drug given that was tested in vitro	20	0	11	9†

*Reprinted from ref 21 with permission of the publisher.

†Acquisition of resistance (increase in the area under the curve) was more common when the patient received the specific drug tested in vitro ($P = 0.03$).

TABLE 3.—Published series of clinical correlations with HTCA

Year of publication	Tumor types	No. of trials	True-positive in vivo/total positive in vitro (%)	True-negative in vivo/total negative in vitro (%)	Cloning method[*]	Investigator (ref No.)
1978	Ovarian, myeloma	32	11/12 (92)	20/20 (100)	H & S	Salmon et al (13)
1981	Ovarian	44	8/11 (73)	33/33 (100)	H & S	Alberts et al (23)
1981	Melanoma	48	12/19 (63)[†]	25/29 (86)	H & S	Meyskens et al (24)
1981	Multiple	123	15/21 (71)	100/102 (98)	H & S	Von Hoff et al (26)
1982	Melanoma	49	10/11 (91)[‡]	38/38 (100)	C & M	Tveit et al (45)
1982	Multiple	36	9/11 (82)	24/25 (96)	H & S	Mann et al (43)
1983	Lung	24	8/12 (67)	12/12 (100)	H & S	Carney et al (29)
1983	Myeloma	33	11/15 (73)	15/18 (83)	H & S	Durie et al (44)
1983	Multiple	246	26/43 (60)	172/203 (85)	H & S	Von Hoff et al (27)
Totals		635	110/155 (71)	439/480 (91)		

[*] H & S = method of Hamburger and Salmon (6); C & M = method of Courtenay and Mills (7).

[†] Mixed responses included as responses.

[‡] Mixed responses and stable disease included as responses.

tive single-agent trial (27), the true-positive rate (60%) and true-negative rate (85%) were both lower than had been reported from the earlier retrospective correlations by the same group (26). A number of technical and logistic difficulties still limit the use of HTCA for selection of chemotherapy. For most tumor types, the limitations in growth rate and cloning efficiency, the lack of effective drugs (as reflected by a high frequency of in vitro resistance), the poor clinical condition of many patients whose tumors are submitted, and clinical decisions for selection of alternative chemotherapy irrespective of assay results would appear to currently preclude routine application of HTCA. Correlations between in vitro sensitivity and clinical response to combination chemotherapy are difficult to evaluate. Clinical studies of ovarian cancer should advance more rapidly than those of other tumor types, since adequate growth is observed frequently, and a number of active drugs are available for testing in vitro and in the clinic. Major efforts at present are directed towards enhancing in vitro growth conditions by the use of specific growth or conditioning factors for specific tumor types such as lung (29) or breast (46) cancer, or by the use of an altered micro-environment (47). It is of importance to realize that tumor cells in a Petri dish are not a tumor in the patient and that many major differences exist. These include the lack of inherent means to activate or inactivate drugs which undergo metabolism. While efforts have been made to include metabolic activation techniques, these can be at best only crude simulations of the in vivo environment.

In Vitro Phase II Trials

While chemosensitivity testing of tumor specimens and clinical correlations of drug sensitivity have provided evidence for validity of HTCA as an assay for measuring correlative drug sensitivity, it is also a good scientific basis for other types of pharmacologic studies. Another major area of application of HTCA has been to new drug development. For example, the assay has been utilized as an adjunct to phase I–II clinical trials of new anticancer drugs. This application has been described as the in vitro phase II trial (21,48). There are two key objectives in the in vitro phase II trial. The first objective is to determine concentrations of a new agent which exhibit significant cytotoxic activity against TCFU. Such data can be of use in relation to phase I clinical trials, since they help to define the plasma concentration-time products which may be required to observe antitumor activity in vivo. The second objective of the in vitro phase II trial is to obtain a preliminary projection of the antitumor spectrum and "response rate" by tumor type for specific new agents. Such information can be of great value in focusing clinical resources with respect to tumor types, which may be anticipated to exhibit clinical sensitivity to a given new agent. Optimally, such data should be obtained against a large battery of human tumors of major histologic types and published prior to the initiation of phase II clinical trials. In vitro phase II studies of mitoxantrone (32), bisantrene (33), and recombinant leukocyte interferons (35) were all published prior to the phase II clinical trials of these agents. Thus, the predictive accuracy for various new agents can be prospectively validated by tumor type. On preliminary assessment, clinical antitumor activity observed to date with mitoxantrone appears to correspond rather closely to that which was predicted from the in vitro phase II trial (32). Another application of the in vitro phase II approach has been to select specific patients for entry in phase I clinical trials. The selection of cancer patients for entry in phase I clinical trials has long been a difficult ethical and logistic problem. Patients are often selected for entry in phase I trials after they have been treated and have failed to respond to a variety of standard agents or if they have tumor types which are known to show inherent resistance (eg, colon or lung cancer) to available agents. Such selections do not maximize the likelihood of observing antitumor activity, and with heavily pretreated patients, do not give a realistic quantitative idea of the toxicity to be anticipated with the given agent. An alternative approach to selection of patients for phase I study, using HTCA results as the basis for selection, was recently reported wth bisantrene and was associated with a surprisingly high objective response rate in phase I (34).

Preclinical Screening

Another important application of HCTA for new drug development is in the initial search for new anticancer drugs. While a detailed discussion of this area of research with HTCA is beyond the scope of this paper, it has been recently discussed elsewhere (14,49). The reason for focus on an in vitro screening procedure for discovery of entirely new drugs, by testing a large number of spontaneous human tumors, is in part because the existing in vivo murine screening system has not been predictive of response in cancer patients in relation to tumor type. In general, the in vivo leukemia screen has identified agents which in a general sense have antiproliferative activity. If there is any specificity to this screening procedure, it likely is to bone marrow stem cells, which are generally related in drug-responsiveness to leukemia cells. It is therefore understandable that agents with tissue-specific mechanisms of action (eg, tamoxifen) were not identified with a leukemia screening procedure but required more tumor-specific models for detection of antitumor activity. The National Cancer Institute (NCI) has recently launched a major effort to test the HTCA as a means for identifying novel antitumor agents which may have been missed with the leukemia prescreen. All experiments were conducted with the investigators blinded as to the compounds they were testing. The initial objectives of the NCI study were to assess the feasibility and reproducibility of HTCA for new drug screening and to enhance its quality control. After the developmental studies were completed, the next major objective was to determine whether HTCA could detect new agents which were missed in the in vivo P388 murine prescreen. Initial results from the NCI study have recently been reported (50). While the results of the NCI study are still quite preliminary, they indicate that HTCA can detect most clinically active agents (with the exception of a few requiring bioactivation and a few antimetabolites). It also correctly detected as negatives a variety of simple sugars, amino acids, and salts. Screening of P388-negative compounds in HTCA was recently initiated by the NCI, and a small proportion of P388-negative compounds appear to exert antitumor activity in vitro in HTCA (50). A major long-term goal of the NCI study is to select novel agents identified with HTCA screening for advancement to clinical trial. This latter test represents the culmination of the experiment, and is intended to establish whether an in vitro screen can identify unique and effective compounds which would have been missed with conventional in vivo screening techniques. Discovery of even a few highly effective agents with this procedure would more than recoup the cost of the NCI experiment.

CONCLUDING REMARKS

Viewed overall, the HTCA provides a potentially important new tool with which to evaluate cancer biology and clinical applications of chemotherapy as well as serving as an aid in drug development. The data obtained on acquisition of drug resistance to specific classes of agents are consistent with prior observations in animal tumor systems, but represent one of the first direct measurements of this phenomenon in cancer patients. It will be of interest to determine whether acquisition of pleiotropic multidrug resistance (51) can also be documented for human tumors grown in HTCA. HTCA may also prove useful for assessing genetic mechanisms of drug resistance (52). It would be desirable to have a short-term assay which could yield quantitatively and qualitatively similar results to those obtained with clonogenic assay. Unfortunately, systematic comparison of short-term dye-exclusion assays and thymidine-labeling procedures has proven inadequate because they lack the time scale required to allow both complete recovery from sublethal damage and complete expression of potentially lethal damage to manifest themselves (53). The relative infrequency of marked in vitro sensitivity to pharmacologically achievable concentrations of many of the currently available anticancer drugs in HTCA is also concordant with clinical experience in a variety of solid tumors. Clinical correlations summarized in this paper provide strong evidence that the HTCA is able to predict clinical chemosensitivity with reasonable accuracy and drug resistance with a high degree of accuracy. While these findings are analogous to those obtained with antibiotic sensitivity testing for bacterial infections, there are a number of difficulties in routinely applying the colony assay for chemosensitivity testing of human tumors. Cloning efficiencies have been low and have limited the extent of experimentation feasible (and the survival curves to one to two logs). Critical review (41) and efforts to improve HTCA procedures are therefore clearly indicated. Applications of HTCA to clinical chemosensitivity testing and new drug development are obviously still in their infancy and are likely to undergo continued developmental and evolutionary change. Improved methods are needed to prepare better single-cell suspensions from solid tumors and to enhance tumor-colony growth. Criteria for drug sensitivity are empiric and subject to change. Randomized clinical studies will be needed to determine whether the assay identifies patients more likely to respond to treatment in a general sense or specifically responsive to the selected agent. While caution regarding clinical applications is warranted, the data obtained to date provide a reasonable

basis for application of HTCA to basic and clinical cancer research. The pace of research has already quickened and a large number of centers have become engaged in this pursuit. Effective implementation of HTCA for chemosensitivity testing is, of course, dependent also on the availability of effective anticancer drugs. The available agents at present are first-generation drugs and lack efficacy for a number of common tumor types (ie, lung, colon, and pancreas). In that regard, HTCA for new drug screening and development may prove to be its most useful future application.

REFERENCES

1. PARK CH, BERGSAGEL DE, and MCCULLOCH EA. Mouse myeloma tumor stem cells: a primary cell culture assay. JNCI 46:411-422, 1971.
2. BRUCE WR, MEEKER BE, and VALERIOTE FA. Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. JNCI 37:233-245, 1966.
3. STEELE GG. Growth and survival of tumour stem cells. *In* Growth Kinetics of Tumors, Oxford, Clarendon Press, 1977, pp 217-262.
4. OGAWA M, BERGSAGEL DE, and MCCULLOCH EA. Chemotherapy of mouse myeloma: quantitative cell culture predictive of response in vivo. Blood 41:7-15, 1973.
5. HAMBURGER AW, and SALMON SE. Primary bioassay of human myeloma stem cells. J Clin Invest 60:846-854, 1977.
6. ______. Primary bioassay of human tumor stem cells. Science 197:461-463, 1977.
7. COURTENAY FD, and MILLS J. An in vitro colony assay for human tumors grown in immune-suppressed mice and treated in vivo with cytotoxic agents. Br J Cancer 37:261-268, 1978.
8. HAMBURGER AW, SALMON SE, KIM MB, ET AL. Direct cloning of human ovarian carcinoma cells in agar. Cancer Res 38:3438-3443, 1978.
9. PAVELIC ZP, SLOCUM HK, RUSTUM YM, ET AL. Colony growth in soft agar of human melanoma, sarcoma and lung carcinoma cells disaggregated by mechanical and enzymatic methods. Cancer Res 40:2160-2164, 1980.
10. HAMBURGER AW, WHITE CP, and TENCER K. Effect of enzymatic disaggregation on proliferation of human tumor cells in soft agar. JNCI 68:945-948, 1981.
11. SALMON SE, ALBERTS DS, MEYSKENS FL, ET AL. Clinical correlations of in vitro drug sensitivity. *In* Cloning of Human Tumor Stem Cells, New York, Alan R. Liss, Inc, 1980, pp 223-245.
12. ALBERTS DS, SALMON SE, CHEN HSG, ET AL. Pharmacologic studies of anticancer drugs using the human tumor stem cell assay. Cancer Chemother Pharmacol 6:253-264, 1981.
13. SALMON SE, HAMBURGER AW, SOEHNLEN B, ET AL. Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs. N Engl J Med 298:1321-1327, 1978.
14. SALMON SE. Cloning of Human Tumor Stem Cells. New York, Alan R. Liss, Inc, 1980.
15. SALMON SE, LIU R, HAYES C, ET AL. Usefulness of abrin as a positive control for the human tumor clonogenic assay. IND, Dec 1983. In press.
16. KRESSNER BE, MORTON RRA, MARTENS AE, ET AL. Use of an image analysis system to count colonies in stem cell assays of human tumors. *In* Cloning of Human Tumor Stem Cells. New York, Alan R. Liss, Inc, 1980, pp 179-193.
17. SALMON SE. Morphologic studies of tumor colonies. *In* Cloning of Human Tumor Stem Cells. New York, Alan R. Liss, Inc, 1980, pp 135-152.
18. ALLEY MC, UHL CB, and LIEBER MM. Improved detection of drug cytotoxicity in the soft agar colony formation assay through use of a metabolizable tetrazolium salt. Life Sci 31:3071-3078, 1982.
19. MOON TE. Quantitative and statistical analysis of the association between in vitro and in vivo studies. *In* Cloning of Human Tumor Stem Cells. New York, Alan R. Liss, Inc, 1980, pp 209-221.
20. MOON TE, SALMON SE, WHITE CS, ET AL. Quantitative association between the in vitro human tumor stem cell assay and clinical response to cancer chemotherapy. Cancer Chemother Pharmacol 6:211-218, 1981.
21. SALMON SE. Applications of the human tumor stem cell assay to new drug evaluation and screening. *In* Cloning of Human Tumor Stem Cells. New York, Alan R. Liss, Inc, 1980, pp 291-312.
22. ALBERTS DS, SALMON SE, CHEN HSG, ET AL. Predictive chemotherapy of ovarian cancer using an in vitro clonogenic assay. Lancet 2:340-342, 1980.
23. ALBERTS DS, CHEN HSG, SALMON SE, ET AL. Chemotherapy of ovarian cancer directed by the human tumor stem cell assay. Cancer Chemother Pharmacol 6:279-285, 1981.
24. MEYSKENS FL, MOON TE, DANA B, ET AL. Quantitation of drug sensitivity by human metastatic melanoma colony forming units. Br J Cancer 44:787-797, 1981.
25. MEYSKENS FL, LOESCHER L, MOON TE, ET AL. A prospective trial of single agent chemotherapy for metastatic malignant melanoma directed by in vitro colony survival in a clonogenic assay. Proc Am Assoc Cancer Res 24:143, 1983.
26. VON HOFF DD, CASPER J, BRADLEY E, ET AL. Association between human tumor colony forming assay results and response of an individual patient's tumor to chemotherapy. Am J Med 70:1027-1032, 1981.
27. VON HOFF DD, CLARK GM, STOGDILL BJ, ET AL. Prospective clinical trial of a human tumor cloning system. Cancer Res 43:1926-1931, 1983.
28. OZOLS RF, YOUNG RC, SPEYER JL, ET AL. Phase I and pharmacological studies of adriamycin administered intraperitoneally to patients with ovarian cancer. Cancer Res 42:4265-4269, 1982.
29. CARNEY DH, BRODER L, EDELSTEIN M, ET AL. Experimental studies of the biology of human small cell lung cancer. Cancer Treat Rep 67:27-35, 1983.
30. AHMAN FR, MEYSKENS FL, MOON TE, ET AL. In vitro chemosensitivities of human tumor stem cells to the phase II drug 4'-(9-acridinylamino)methanesulfon-*m*-anisidide and prospective in vivo correlations. Cancer Res 42:4495-4498, 1982.
31. SALMON SE, and VON HOFF DD. In vitro evaluation of anticancer drugs with the human tumor stem cell assay. Semin Oncol 8:377-385, 1981.
32. VON HOFF DD, COLTMAN CA, and FORSETH B. Activity of mitoxantrone in a human tumor cloning system. Cancer Res 41:1853-1855, 1981.
33. ______. Activity of 9-10 anthracenedicarboxaldehyde bis ((4,5-dihydro-1*H*-imidazol-2yl)hydrazone) dihydrochloride (CL-216,942) in a human tumor cloning system. Cancer Chemother Pharmacol 6:141-144, 1981.
34. ALBERTS DS, MACKEL C, POCELINKO R, ET AL. Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis ((4,5-dihydro-1*H*-imidazol-2-yl)hydrazone) dihydrochloride with correlative in vitro human tumor clonogenic assay. Cancer Res 42:1170-1175, 1982.
35. SALMON SE, DURIE BGM, YOUNG L, ET AL. Effects of cloned human leukocyte interferons in the human tumor stem cell assay. J Clin Oncol 1:217-225, 1983.
36. SALMON SE. Background and overview. *In* Cloning of Human Tumor Stem Cells. New York, Alan R. Liss, Inc, 1980, pp 3-14.
37. CARNEY DN, GAZDAR AR, BUNN PA, JR, ET AL. Demonstration of the stem cell nature of clonogenic tumor cells from lung cancer patients. Stem Cells 1:149-164, 1981.
38. THOMSON SP, and MEYSKENS FL, JR. Method for measurement of self-renewal capacity of clonogenic cells from biopsies of metastatic human malignant melanoma. Cancer Res 42:4606-4613, 1982.
39. BUICK RN, and MACKILLOP WJ. Measurement of self-renewal in culture of clonogenic cells from human ovarian carcinoma. Br J Cancer 44:349-355, 1981.

40. MACKILLOP WJ, CIAMPI A, TILL JE, ET AL. A stem cell model of human tumor growth: implications for tumor cell clonogenic assays. JNCI 70:9-16, 1983.
41. SELBY P, BUICK RN, and TANNOCK I. A critical appraisal of the human tumor stem cell assay. N Engl J Med 308:129-134, 1983.
42. LUDWIG R, ALBERTS DS, MILLER TP, ET AL. Schedule dependency of anticancer drugs in the human tumor stem cell assay. Cancer Chemother Phamacol, 1983. In press.
43. MANN BD, KERN DH, GIULIANO AE, ET AL. Clinical correlations with drug sensitivities in the clonogenic assay. Arch Surg 117:33-36, 1982.
44. DURIE BGM, YOUNG L, and SALMON SE. Human myeloma stem cell culture: interrelationships between drug sensitivity, cell kinetics and patient survival duration. Blood 61:929-934, 1983.
45. TVEIT KM, FODSTAD O, LOTSBERG J, ET AL. Colony growth and chemosensitivity in vitro of human melanoma biopsies. Relationship to clinical parameters. Int J Cancer 29:533-538, 1982.
46. HUG V, SPITZER G, DREWINKO B, ET AL. Improved culture conditions for the in vitro growth of human breast tumors. Proc Am Assoc Cancer Res 24:35, 1983.
47. VON HOFF DD, and HOANG M. A new perfused capillary cloning system to improve cloning of human tumors. Proc Am Assoc Cancer Res 24:310, 1983.
48. SALMON SE, MEYSKENS FL, ALBERTS DS, ET AL. New drugs in ovarian cancer and malignant melanoma: in vitro phase II screening with the human tumor stem cell assay. Cancer Treat Rep 65:1-12, 1981.
49. SALMON SE. Application of the human tumor stem cell assay in the development of anticancer therapy. *In* Cancer: Achievements, Challenges and Prospects for the 1980's. New York, Grune and Stratton, 1980, vol 2, pp 33-43.
50. SHOEMAKER RH, WOLPERT-DEFILIPPES MK, MAKUCH RW, ET AL. Use of the human tumor clonogenic assay for new drug screening. Proc Am Assoc Cancer Res 24:311, 1983.
51. LING V. Genetic basis of drug resistance in mammalian cells. *In* Drug and Hormone Resistance in Neoplasia (Bruchovsky N, and Goldie JH, eds). Boca Raton, FL, CRC Press, 1982.
52. TRENT JM, and SALMON SE. Clonal expression of drug resistance and genetic heterogeneity in spontaneous human tumors. Proc Am Assoc Cancer Res 24:282, 1983.
53. RUPNIAK HT, DENNIS LY, and HILL BT. An intercomparison of in vitro assays for assessing cytotoxicity after a 24-hour exposure to anticancer drugs. Tumori 69:37-42, 1983.

Liposomes as Drug Carriers in Cancer Therapy

John N. Weinstein[1,*]

Liposomes, microscopic particles composed of lipid bilayer membranes, can carry water-soluble drugs in their aqueous compartments and lipid soluble drugs in their bilayers. Over the last 10 years, they have been studied more intensively than any other type of drug carrier. The first impulse, as with other pharmaceuticals, has been to inject them iv. However, since they do not leave the vascular system readily except in the sinusoids of liver and spleen, many of the projected applications to nonhematogenous cancers are unlikely to succeed. Nonetheless, the last few years have brought a great increase in sophistication with respect to the physical chemistry, biochemistry, and pharmacology of liposomes. Routes other than the vascular are being explored, and iv injection is being exploited for access to targets within the vascular space. The most likely clinical applications to be successful soon are in parasitology and immunology; practical applications in oncology are distinctly possible, but still speculative. [Cancer Treat Rep 68:127–135, 1984]

Liposomes are microscopic particles composed of one or more phospholipid bilayer membranes. Naturally occurring liposomes probably played a major role in prebiotic evolution (1), and some would say that their function in distinguishing an inside from an outside preceded even the nucleotide-based code. As objects of scientific study, however, liposomes are younger than *Cancer Treatment Reports*. The study of liposomes began with Bangham et al (2,3) in the mid-1960s when they added water to dried phospholipids on a microscope slide and were "beguiled by the process" in which bilayers peeled from the slide as writhing snakes or rounded projections, finally budding off to form particles. These events could be seen in full spectral colors when viewed through crossed polarizers. Bangham noticed that the particles changed shape in response to osmotic stresses and guessed that they formed closed, discrete, membranous bodies. That supposition proved correct, hence the term "liposome" (lipid + body).[2]

Since 1965, research on liposomes has progressed along several tracks. The first track has been physical chemical; the stacks of hydrated lipid bilayers (smectic mesophase) which make up spontaneously formed liposomes obey colloid chemical rules which are now slowly coming into focus. The second track has been cell biological; liposomal bilayers can be thought of as models for, or components of, cell membranes. This biological connection has, in fact, motivated much of the physical chemical study of lipid bilayers. The cell biologist may also consider the liposome as a tool for inserting new components into cell membranes or for injecting water-soluble materials into the cytoplasm of the cell. That sort of endeavor brings us closer to the third track, the use of liposomes as carriers of drugs and biologicals in vivo for medical applications.

Almost from the beginning, some of Bangham's disciples began to think of liposomes as microcapsules capable of carrying water-soluble agents in their aqueous compartments and lipid-soluble agents in their bilayers. Initially, the focus was on liposome-entrapped enzymes for replacement therapy in genetic deficiency diseases. However, the aims were broadened to include a considerable emphasis on cancer. In some cases, liposomes have been studied as drug carriers without any firm rationale, simply in the hope that the pharmacokinetic rules of drug delivery would somehow change to advantage if the agent were encapsulated in a liposome. More often, the rationale for introduction of liposomes has been one of those to be discussed here shortly, after brief introductory sections on the characteristics of liposomes and their interactions with cells. This survey will then conclude with a list of the problems confronting liposome pharmacology and a few comments on the prospects for practical

---

[1] Laboratory of Mathematical Biology, Division of Cancer Biology and Diagnosis, National Cancer Institute, Bethesda, MD.

[2] The terms "liposome" and "lipid vesicle" have occasionally been used for multilamellar and unilamellar particles, respectively, but the two terms will be used interchangeably here.

[*] *Reprint requests to:* John N. Weinstein, MD, Laboratory of Mathematical Biology, Division of Cancer Biology and Diagnosis, National Cancer Institute, Bldg 10, Rm 4B54, National Institutes of Health, Bethesda, MD 20205.

application to cancer.

This is not intended either as a comprehensive review or as a balanced assessment of credit for ideas in what is often a rather muddy literature. For a more comprehensive treatment of the subject and fuller citation of the literature, the reader is referred to a recent review by the author (4). Several recent books (5-9) and reviews (10-15) have also dealt with various aspects of the subject. We will focus here on the pharmaceutical applications of liposomes; in the end, however, liposomes may affect our approach to cancer more by their roles in basic biology and immunology than by their use as pharmaceuticals.

PHYSICAL CHEMISTRY OF LIPOSOMES

Types of Liposomes

Figure 1 shows the three major types of liposomes, multilamellar large vesicles (MLV), small unilamellar vesicles (SUV), and large unilamellar vesicles (LUV). MLV form spontaneously when appropriate lipids are hydrated. They range in size from about 80 nm to about 10 µm, and there is considerable size heterogeneity in any given preparation. More homogeneous populations can be obtained by filtering the crude preparation. SUV are formed from MLV by an input of energy, generally ultrasonic. In principle, SUV can be as small as 20 nm in diameter, this lower limit being set by strains in packing of the lipid molecules. However, liposomes for use in vivo have almost always contained large amounts of cholesterol, and such SUV are at least 50 nm in diameter. SUV have a high surface-to-volume ratio, hence they carry little water-soluble drug per milligram of lipid. The third type of liposome, LUV, has come into prominence only in the last few years. The most frequently used method for their preparation is "reverse-phase evaporation" (16). LUV may have one bilayer, as the name suggests, but may also in practice be oligolamellar structures, depending on the exact conditions during their production. Again, size heterogeneity of preparations can be reduced by filtration. Representative values for captured aqueous volume in SUV, MLV, and LUV are 0.5, 4, and 11 µl/mg of lipid, respectively. Typical efficiencies of encapsulation for small water-soluble molecules are 1%, 10%, and 40%, respectively, for the three types. Macromolecules can be trapped effectively in MLV and LUV, but not in SUV.

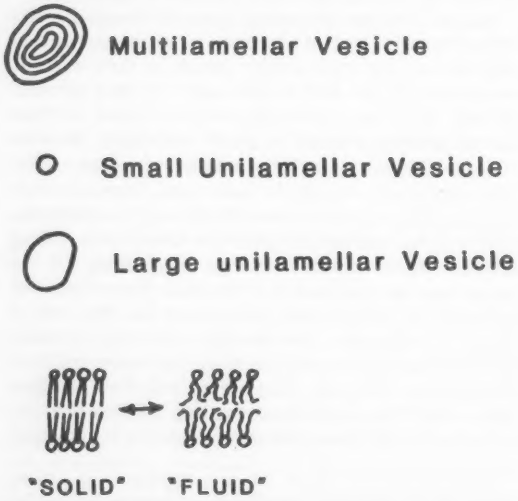


FIGURE 1.—Three types of liposomes. Each line represents a lipid bilayer. Also shown schematically is the phase transition from "solid" to "fluid" state of the bilayer (ref 12).

Liposomal Lipids

The lipids which form bilayers (and hence liposomes) have two hydrocarbon chains and a hydrophilic head group. In geometric terms there is a rough balance between the chains and head group so that the molecules can pack side-by-side as cylinders to form flat, extended structures. Lipids in which the head group predominates (eg, some detergents) tend instead to form structures which curve around on themselves (micelles); lipids in which the hydrocarbon tails predominate fail to hydrate entirely or else curve in the opposite direction to form inverted micelles (in which the head groups point inward toward an aqueous center and the chains extend outward). Common bilayer-forming phospholipids are the phosphatidylcholines (old name: lecithin), sphingomyelins, phosphatidylserines, and phosphatidylethanolamines. Charged lipids (to change the surface properties) or cholesterol (to stabilize the bilayer structure against attack by serum components) can be added to the basic bilayer structure.

The lipid class designation (eg, phosphatidylcholine) specifies the head group, but not the length or degree of unsaturation of the hydrocarbon chains. The chains usually have 14-18 carbon atoms and are either completely saturated or have a double bond in the 9-10 position. For each pure type of phospholipid, there is a temperature (T_m) at which its chains undergo a transition from a well-ordered, solid-like arrangement to a much more fluid one. That is, the lipid bilayer melts in much the same sense as does ice. For unsaturated chains, T_m is generally $< 0°C$; for fully saturated chains of 14 or 16 carbons, T_m is near the physiologic range. That fact forms the basis for "temperature-sensitive liposomes," which will be discussed later.

The oil-water partition coefficient and charge largely determine how drugs associate with liposomes. Water-soluble agents are entrapped in the aqueous compartment(s) by forming the liposomes in the presence of dissolved drug. For small molecules, the trapping is directly determined by the ratio of internal to external aqueous volume. Lipid-soluble agents partition into the bilayer to differing degrees. Amphiphilic drugs (eg, the anthracyclines) can be stabilized in liposomes by complexation with other small molecules or with charged lipids.

INTERACTION WITH CELLS

Liposomes can interact with cells in several different ways (17), as shown schematically in figure 2. Adsorption can be either nonspecific or mediated by specific ligands such as the antibodies, hormones, and lectins; it may or may not lead secondarily to endocytic incorporation of the liposomes and their contents into the cell. Lipid exchange is a process in which lipid molecules transfer between the cell membrane and the outermost monolayer of the liposome, without any association of the liposome as a body with the cell.

Endocytosis is by far the most important form of interaction. "Professional" phagocytes take up liposomes avidly, although it is not clear what receptors or signal mechanisms are responsible. Nonphagocytic cells also appear to endocytose liposomes, although to a much lesser extent. It has recently been reported that small negatively charged vesicles are taken up via coated pits, delivered to uncoated vesicles, and carried to the trans-golgi region of the cell (18). These observations correlate well with another recent report (19) that the endocytic uptake is selective for small vesicles (< 100 nm in diameter). Thus, SUV may be more efficient for delivery into cells than the larger forms, even if they carry less agent per unit of lipid. Once taken into the endosomal apparatus, the constant question is whether and how the materials can escape to affect targets in the cytoplasm and nucleus.

Fusion of liposomes with the cell membrane would be expected to insert any lipid or protein originally in the liposome bilayer into the membrane. Water-soluble contents of unilamellar vesicles would be injected into the cytoplasm; water-soluble contents of multilamellar forms would enter the cytoplasmic compartment still sequestered in lamellar structures having one fewer bilayer

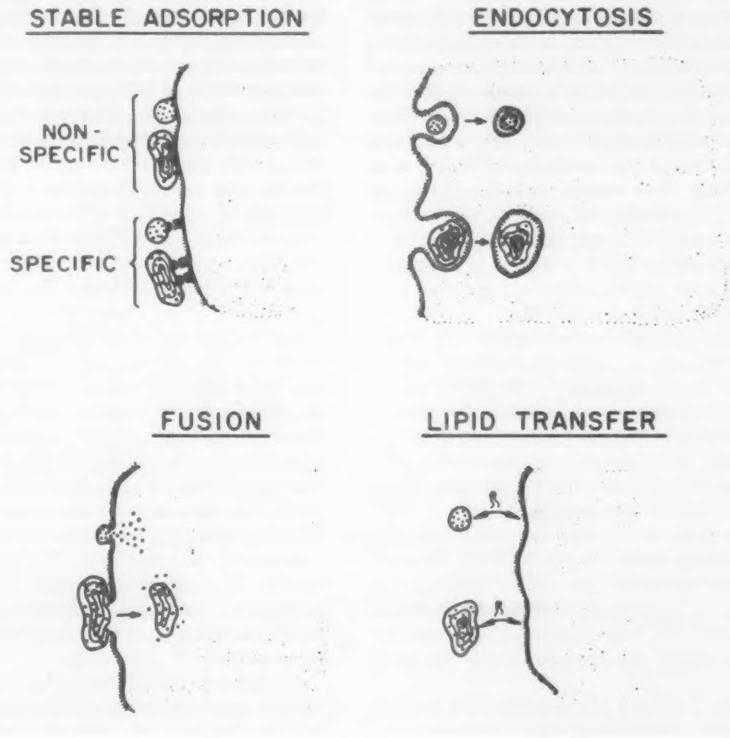


FIGURE 2.—Possible mechanisms of interaction between liposomes and cells. Both unilamellar and multilamellar vesicles are shown. Binding can be either nonspecific (mediated by physical chemical forces) or specific (mediated by a high-affinity ligand) (ref 17).

than the original MLV. Unfortunately, however, there is little or no spontaneous fusion between cell membranes and the usual types of liposomes. That has doomed many of the early, fond hopes for use of liposomes in cell biology and medicine. There is now emerging a second generation of techniques (4) using nonspecific perturbants such as polyethylene glycol or glycerol to promote entry either through the plasma membrane or through endosomal membranes (it is unclear which). Viral coat proteins have also been inserted into liposomes as a mechanism for promoting entry into the cell. None of these techniques is currently feasible in vivo.

RATIONALE FOR USE OF LIPOSOMES

Numerous possible reasons for use of liposomes (or other carriers) in vivo have been advanced, some of them more compelling than others.

(*a*) *Sequestration of the liposome as a particle.*—Iv injected liposomes have been reported to accumulate outside of injured vessels in myocardial infarctions. It has been hoped that vesicles would also accumulate outside of neovasculature or necrotic vessels in tumors. Despite scattered suggestions, there is no good evidence that usable extravasation of this sort takes place. One limiting factor may be the low blood flow in areas of necrosis.

(*b*) *Prolonged circulation.*—Liposomes circulate in the bloodstream much longer than most free drugs. Clearance times, generally a matter of hours, can be increased by decreasing the size of the vesicle and including negatively charged lipids. Slow release can certainly increase the potency of cell-cycle dependent drugs, but toxic effects tend to increase in tandem; therapeutic indices appear difficult to improve by this strategy. As other techniques for continuous infusion improve, this reason for use of liposomes becomes less convincing.

(*c*) *Protection of liposome contents against the host.*—Encapsulation can protect liposome contents against immunologic and enzymatic attack in the physiologic environment. It should also provide a favorable milieu in which to preserve the potency of labile agents until they reach their targets. The platinum compounds, for example, could be kept in the circulation in a medium of any desired chloride concentration and pH.

(*d*) *Protection of the host against liposome contents.*—Liposomes do not accumulate in normal heart muscle or kidney tubules to the extent that many free drugs do. Hence, they may be useful as carriers of cardiotoxic and nephrotoxic agents. They have been studied extensively as a means of reducing the cardiotoxicity of anthracyclines (20,21).

(*e*) *Amplification.*—Only a few molecules of a drug can be directly coupled to small molecular carriers, such as the antibodies or hormones. A target-directed liposome, on the other hand, can carry hundreds to thousands of drug or radionuclide molecules.

(*f*) *Circumvention of cell membrane permeability barriers.*—Fusion has long been considered a means of overcoming transport-associated drug resistance. However, as stated in the last section, spontaneous fusion is at best low-level in vitro and is currently out of the question in vivo. Any such circumvention of transport limitations would depend on endocytosis and subsequent escape from the endosomal apparatus.

(*g*) *Delivery of drugs designed specifically for endocytic uptake.*—To the present time, drugs encapsulated in liposomes have almost always been those effective in free form against the target disease. In the long run, that is clearly not the right strategy, since the pharmacological rules change dramatically when a drug is entrapped in liposomes. For example, methotrexate-γ-aspartate (which efficiently inhibits dihydrofolate reductase) proved ineffective in vitro in free form because of poor transport into cells. When trapped in liposomes (22), it was taken up by endocytosis and escaped into the cytoplasm to bind the enzyme. Escape may have depended on the fact that methotrexate is a weak anion and, as such, would tend to partition out of the acidic endosomal environment. Agents designed for effect after endocytosis have been termed "endodrugs" (D. Papahadjopoulos, personal communication). Ideally, in this context, one would want a methotrexate derivative which was unable to cross the plasma membrane in free form, but able to pass efficiently from endosome to cytoplasm and bind dihydrofolate reductase; it would be unable to leave the cell because of extra amino acid residues (eg, the aspartate) attached by noncleavable bonds. The potency of such an agent when encapsulated (and lack of potency if released from the vesicles) would translate into an improved therapeutic index if the vesicles could be designed for endocytosis preferentially by the target cells. *There* is a challenge for the chemist and the cell biologist!

(*h*) *Confinement to an anatomic compartment.*—Liposomes are too large to pass efficiently across epithelial and endothelial cell layers or across intact basement membranes (23). Therefore, their distribution is determined by the route of administration. Prime routes for consideration are peritoneal, for treatment of ovarian carcinoma locally or passage to the mediastinal lymph nodes (24); subcutaneous, for tracking to regional nodes (25,26); pulmonary, by instillation or inhalation (27); and intrathecal, for treatment of central nervous system tumors. In contexts other than that of cancer, intra-articular and oral routes have also been tried. The vascular compartment is, of course, appropriate if the target is intravascular.

(*i*) *Ligand-mediated "targeting."*—Several classes of ligands have been attached to liposomes as a means of selectively binding them to tumor cells: sugars, lectins, peptide hormones, haptens, antibodies, and other proteins (4). To summarize a large body of work in a few sentences, it has not generally proved difficult to bind liposomes selectively to target cells in vitro, and selective association has also been attainable in vivo when the liposomes were injected into the anatomic compartment containing the target cells. However, it became evident quite early that binding to the cell surface did not imply entry of liposome contents into the cell (28). Entry appears to depend on endocytosis (29), not fusion with the plasma membrane, and the amount endocytosed depends on the particular cell-surface determinant bound, the manner in which it is bound, and the cell type. Furthermore, entry into the endosomal apparatus does not guarantee escape of the liposomal agent into the cytoplasm or nucleus. As already discussed, that process may depend on a variety of factors, including the titration characteristics of the drug. In the last few years, more than a dozen techniques have been developed for covalently linking proteins (generally antibodies) to liposomes (4). The most successful techniques have used heterobifunctional cross-linking reagents, which minimize coupling of ligand to ligand and liposome to liposome (30,32). Cellular studies using these methods are beginning in earnest, and we can expect to have a much greater understanding of ligand-mediated delivery within the next 2 or 3 years.

(*j*) *Physical "targeting."*—Some characteristic of the environment is used either to direct the liposome to a particular location or to cause selective release of its contents there. The first such approach was the "temperature-sensitive" liposome (33,34). In the presence of serum components, principally the lipoproteins, liposomes release their contents rapidly and completely at T_m. By using a mixture of lipids with T_m at about 42°C, the liposomes can be designed to release a drug preferentially in a capillary bed (ie, in a tumor) subjected to moderate local hyperthermia (fig 3). Temperature-sensitive liposomes have achieved selectivities of greater than tenfold between heated and nonheated tumors in the delivery of methotrexate (35), and qualitatively similar results with cisplatin (36). On the basis of organ-distribution studies, it appears that increases in therapeutic index can be obtained, but no studies to substantiate that proposition directly have yet been done. Current efforts are aimed at improving the liposomes, choosing an appropriate liposome-drug combination, and improving the technology of hyperthermia. This approach to selectivity is limited (as are surgery, radiation therapy, and hyperthermia itself) by the necessity of knowing the location of the tumor; temperature-sensitive liposomes are not an answer to the problem of widespread metastases. The appeal of physical targeting is the lack of certain problems inherent in ligand-mediated targeting: the requirement for a usable determinant on the tumor cell; the sequestration of target cells behind endothelial or epithelial barriers; modulation and heterogeneity in the expression of cell surface determinants; immunogenicity of ligand-bearing liposomes and tendency for them to be removed from the circulation quickly; limitation in the number of effector molecules which can be bound to cells; and the requirement in many cases for efficient endocytic uptake and es-

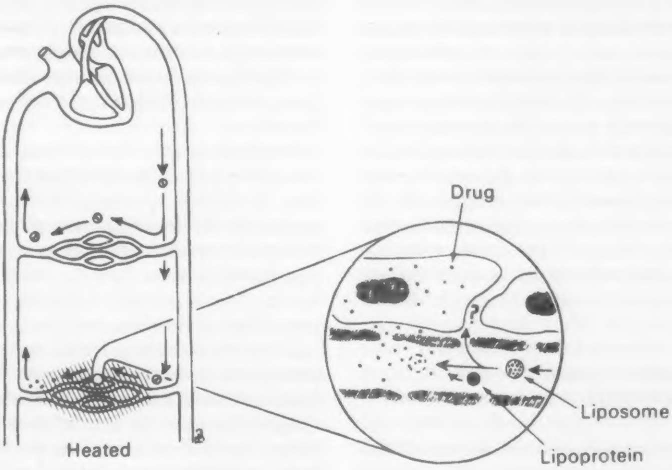


FIGURE 3.—Schematic view of drug release from temperature-sensitive liposomes in the capillaries of a tumor heated to the phase transition temperature. Serum components, principally the lipoproteins, induce rapid release, and drug equilibrates with the extracellular space. This physical targeting does not require that liposomes be able to leave the capillaries or interact directly with the tumor cells (ref 34).

cape of the entrapped agent to the cytoplasm. Most of these problems are also encountered with ligand-linked toxins or drugs; liposomes provide amplification in exchange for difficulty in exiting from the compartment of administration. It has also been suggested that weakly anionic drugs, such as methotrexate, could be made to leak out of liposomes preferentially in low pH regions of tumors (34). Likewise, liposomes made with pH-sensitive lipids have been made for selective release at low pH (37), and magnetic liposomes have been considered as means of localization. None of these physical methods could be useful clinically without considerably more developmental work.

(*k*) *Natural targeting.*—"Reticuloendothelial" phagocytes remove a large percent of injected liposomes from the circulation; hence, they are "natural" targets. Because of its simplicity, this is the most persuasive rationale for use of liposomes and the most likely to bear fruit in cancer chemotherapy in the near future. The archetypal studies of natural targeting have been done in parasitology (reviewed in ref 38). The Leishmania parasite is taken up by, and resides in, the same phagocytic cells that remove liposomes from the bloodstream. Liposome-encapsulated anti-Leishmanial drugs are therefore delivered directly to the host cells, in fact, directly to the parasitic vacuoles within those cells. Dramatic increases in therapeutic index have been achieved. Extrapolating to cancer, one would look for phagocytic tumor cells accessible to a site of administration of the liposomes. The concept can also be reversed: macrophage activating factor, muramyl dipeptide, and muramyl tripeptide phosphatidylethanolamine (a lipophilic derivative) have been selectively delivered to monocytes as a means of recruiting them to destroy tumors cells (39-41). The cells appear to phagocytose liposomes within the bloodstream, then migrate into the tissues where they function as activated macrophages. This approach gains power from the apparent ability of activated macrophages to recognize and kill tumor cells of many sorts, with no apparent requirement for (currently recognized) tumor antigens. An important limitation, as with other attempts to use host defenses against tumors, is the low tumor loads which can be eliminated. Liposome-encapsulated immune modulators would probably be used in combination with "debulking" modalities.

PROBLEMS AND LIMITATIONS OF LIPOSOMES AS CARRIERS

Having considered the possible reasons for use of liposomes, we next consider the problems.

(*a*) *Uptake in liver and spleen.*—As with other carriers, a considerable percent of iv injected liposomes are removed from the circulation by reticuloendothelial cells in the liver, spleen, and elsewhere. Most important quantitatively are the Kupffer cells of the liver. However, smaller vesicles can apparently pass through the 100-nm pores in the hepatic sinusoids to enter the space of Disse and interact directly with hepatocytes. Liver uptake is a factor against use of highly hepatotoxic drugs in liposomes. Attempts to inhibit liver uptake by preadministration of carbon particles, empty liposomes, dextran sulphate, or methylpalmitate have been only modestly successful. Attempts have been made to turn the liver uptake to advantage by targeting hepatic tumors, but incorporation into the liver as an organ does not imply accumulation in tumor there. In an interesting use of the reticuloendothelial clearing mechanism, liposomes bearing anti-IgG have been used to clear radiolabeled IgG from the circulation to reduce body background prior to gamma camera imaging (42).

(*b*) *Inability to escape the bloodstream.*—The continuous endothelium characteristic of most tissues acts as a barrier to the passage of liposomes. Even an intact basement membrane will do likewise in the presence of a disrupted or fenestrated endothelial cell layer (23). Liposomes are thus unlikely to reach the cells of solid tumors unless (*a*) the vessels are severely damaged or incomplete; (*b*) the liposomes are carried out of the blood by diapedesing cells; or (*c*) the liposomes possess the still unknown mechanisms by which cells pass through endothelia and disrupt basement membranes. Small liposomes can apparently reach the marrow spaces, but with what efficiency is not clear. Perhaps larger liposomes could be used to prevent marrow toxicity in the same way as they may be used to forestall the cardiotoxicity of doxorubicin. There remains a possible loophole with respect to passage of vesicles out of the bloodstream: the available data (23) are for vesicles 60-70 nm in diameter. The results might be different for the smallest vesicles, 20 nm in diameter (ie, made without cholesterol, but made of lipids not susceptible to interaction with serum components).

(*c*) *Disruption by serum components.*—The lipoproteins and some other, unidentified serum component tend to disrupt liposomes. The process can be slowed drastically by incorporation of cholesterol (preferably > 30 mols percent), by use of sphingomyelin or saturated phospholipids below their T_m. There are also naturally occurring "anti-liposome" antibodies (43), but their practical effect on liposome stability in vivo is uncertain. Because of the disruptive forces, many in vivo distribution studies using liposome-associated radiolabels (including most of those done prior to 1980) have monitored something other than the fate of liposomes *as intact structures*. Only recently have the problems in methodology and interpretation been worked out satisfactorily.

(*d*) *Antigenicity and immunogenicity.*—As already noted, antibodies to liposomes and liposomal lipids do occur. The possible problem is compounded for liposomes bearing proteins or polysaccharides on their surfaces. Indeed, one of the most advanced applications of liposomes is their use as carriers of immunogenic moieties in vaccines. Paradoxically, liposomes have also been reported to protect antigenic contents from the immune system. The difference appears to depend on whether the immunogens are within the liposome or exposed on its surface (43).

(*e*) *Toxicity.*—Phosphatidylcholine and cholesterol, usually the major ingredients in liposome formation, are biodegradable, relatively nontoxic, and relatively nonimmunogenic. They are, for example, administered in large amounts over long periods as components of lipid mixtures used for iv feeding. While iv feeding is not benign, it can be managed successfully over long periods. The safety of other liposomal components, such as the charged lipids and sphingomyelin, is in more doubt. Even in the absence of life-threatening toxicity, hematologic changes such as activation of the alternative complement pathway have been described, as has reduction in the phagocytic potential of the reticuloendothelial system when subjected to challenge with other particles. Major toxicological studies are clearly needed and it is vital that they be conducted under well-controlled conditions. The preparations used should be tested for pyrogens and other impurities, they should be protected by antioxidants against lipid peroxidation, their size distribution should be determined, and large particles capable of blocking the microvasculature should in general be excluded. The results of past toxicity studies are hard to interpret because such precautions were not taken. It is also clear that entrapment in a liposome can alter the toxicity of a drug, perhaps in the same direction as its therapeutic effect. For example, encapsulation of an anti-Leishmanial drug increased its toxicity an order of magnitude in animal studies (38). (However, its therapeutic effect increased several hundred-fold, hence, the therapeutic index was still greatly increased.)

(*f*) *Questionable shelf life, instability.*—Large-scale pharmaceutical use of liposomes will require attention to a variety of mundane issues: uniformity of particle size, sterility, stability at temperatures of storage, process scale-up, lipid production costs, and dispensing systems. These issues have only recently been addressed, at least in the published literature.

DISCUSSION

We have focused on the rationale for use of liposomes and on problems which may limit their effectiveness in oncology. The practical experience in applying those principles to human and animal tumors is difficult to summarize succinctly. With respect to animal studies, there have been numerous reports that entrapping a drug in liposomes increased its effect on tumor growth, and there have been several reports on modestly increased therapeutic indices. However, to my knowledge, none of these involved established masses of drug-resistant cells. The animal experiments on therapy are reviewed in references 13 and 44.

Methotrexate, cytarabine, and doxorubicin have been the antitumor drugs most often used, but many other agents have received attention (*see* table 2 of ref 4). It must be emphasized that the current pharmacopoeia is unlikely to include the best choices; pharmacologic requirements for a carrier-associated drug are, a priori, different from those for a free drug. Ideally, the encapsulated agent should be specifically selected or designed for the purpose. Methotrexate-γ-aspartate represents a stage in that rational development; the free form enters cells very poorly, but when liposomes containing methotrexate-γ-aspartate are endocytosed by a cell, the drug (or an active moiety of it) escapes into the cell cytoplasm to exert its toxic effect (22). Macrophage-activating factors and the muramyl peptides (39–41) are also well-suited to administration in liposomes; their targets are cells which *normally* endocytose liposomes in vivo.

The clinical experience with liposomes is sparse. Diagnostic imaging of Indium 111, Iodine 131, and Technetium 99m has been tried on single patients, or very small groups of patients, with a spectrum of tumor types (45,46). In a larger trial (47), 40 patients with breast cancer were injected in the periareolar region with liposomes containing Technetium 99m for gamma camera imaging of lymph nodes. One early study indicated preferential localization of radiolabeled liposomes in tumors (48) after iv injection, but negative results were later obtained in similar studies from the same (49) and another (45,46) group. To my knowledge, therapeutic doses of liposome-entrapped anticancer drugs have not been administered to humans.

Liposomes should be viewed in the context of other types of macromolecular carriers: nucleic acids, synthetic polymers, microcapsules, nanoparticles, albumins, antibodies (monoclonal and otherwise), protein aggregates, hormones, and lectins. In a sense, these carriers represent competing technologies. In another sense, however, development of all of the carriers represents a cooperative enterprise. The scientific base required for one carrier overlaps extensively with that required for the others.

In the biological world, the process of maturation is often progressive and inexorable; in the technological world, false starts, digressions, and prematurity are the norm. For most of its short history, the field of liposome pharmacology has struggled under the handicap of an inadequate scientific base of information. As the required ideas and data become available from cell biology, physiology, immunology, biochemistry, and physical chemistry, we will see whether projected applications in oncology take practical shape. For the present, they remain a matter of speculation.

REFERENCES

1. BANGHAM AD. Membrane models with phospholipids. Prog Biophys Mol Biol 18:29, 1968.
2. BANGHAM AD, and HORNE RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J Mol Biol 8:660, 1964.
3. BANGHAM AD, STANDISH MM, and WATKINS JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 13:238, 1965.
4. WEINSTEIN JN, and LESERMAN LD. Liposomes as drug carriers in cancer chemotherapy. Pharmacol Ther, in press.
5. KNIGHT CG, ed. Liposomes, From Physical Structure to Therapeutic Applications. New York, Elsevier, 1981.
6. LESERMAN LD, and BARBET J, eds. Liposome Methodology in Pharmacology and Cell Biology. Paris, Inserm, 1982.
7. GREGORIADIS G, SENIOR J, and TROUET A, eds. Targeting of Drugs. New York, Plenum Press, 1982.
8. OSTRO M, ed. The Liposomes. New York, Marcel Dekker, 1983.
9. GREGORIADIS G, ed. Liposome Technology. New York, CRC Press, in press.
10. SZOKA F, and PAPAHADJOPOULOS D. Comparative properties and methods of preparation of lipid vesicles (liposomes). Ann Rev Biophys Bioeng 9:465, 1980.
11. FRALEY R, and PAPAHADJOPOULOS D. New generation liposomes: the engineering of an efficient vehicle for intracellular delivery of nucleic acids. Trends Biochem Sci 6:77, 1981.
12. WEINSTEIN JN. Liposomes as "targeted" drug carriers: a physical chemical perspective. Pure Appl Chem 53:2241, 1981.
13. KAYE SB. Liposomes—problems and promise as selective drug carriers. Cancer Treat Rev 8:27, 1981.
14. WEINSTEIN JN. Target-direction of liposomes: four strategies for attacking tumor cells. *In* Rational Basis for Chemotherapy (Chabner B, ed). New York, Liss, p 441.
15. POSTE G. Liposome targeting in vivo: problems and opportunities. Biol Cell 47:19, 1983.
16. SZOKA F, and PAPAHADJOPOULOS D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc Natl Acad Sci USA 75:4194, 1978.
17. PAGANO RE, and WEINSTEIN JN. Interactions of liposomes with mammalian cells. Ann Rev Biophys Bioeng 7:435, 1978.
18. STRAUBINGER RM, HONG K, FRIEND DS, ET AL. Endocytosis of liposomes and intracellular fate of encapsulated molecules: encounter with a low pH compartment after internalization in coated vesicles. Cell 32:1069, 1983.
19. MACHY P, BARBET J, and LESERMAN LD. Differential endocytosis and T and B lymphocyte surface molecules evaluated with antibody-bearing fluorescent liposomes containing methotrexate. Proc Natl Acad Sci USA 79:4148, 1982.
20. JULIANO RL, and STAMP D. Pharmacokinetics of liposome-encapsulated anti-tumor drugs. Biochem Pharmacol 27:21, 1978.
21. FORSSEN EA, and TOKES ZA. In vitro and in vivo studies with adriamycin liposomes. Biochem Biophys Res Commun 91:1295, 1979.
22. HEATH TD, MONTGOMERY JA, PIPER JR, ET AL. Antibody-targeted liposomes: increase in specific toxicity of methotrexate-γ-aspartate. Proc Natl Acad Sci USA 80:1377, 1983.
23. POSTE G, BUCANA C, RAZ A, ET AL. Analysis of the fate of systemically administered liposomes and implications for their use in drug delivery. Cancer Res 42:1412, 1982.
24. PARKER RJ, PRIESTER ER, and SIEBER SM. Comparison of lymphatic uptake, metabolism, excretion, and biodistribution of free and liposome-entrapped ^{14}C-cytosine β-D-arabinofuranoside following intraperitoneal administration to rats. Drug Metab Dispos 10:40, 1982.
25. SEGAL AW, GREGORIADIS G, and BLACK CDV. Liposomes as vehicles for the local release of drugs. Clin Sci Mol Med 49:99, 1975.
26. KHATO J, PRIESTER ER, and SIEBER SM. Enhanced lymph node uptake of melphalan following liposomal entrapment and effects on lymph node metastasis in rats. Cancer Treat Rep 66:517-527, 1982.
27. MCCULLOUGH HN, and JULIANO RC. Organ-selective action of an anti-tumor drug: pharmacologic studies of liposome-encapsulated beta-cytosine arabinoside administered via the respiratory system of the rat. JNCI 63:727, 1979.
28. WEINSTEIN JN, BLUMENTHAL R, SHARROW SO, ET AL. Antibody-mediated targeting of liposomes: binding to lymphocytes does not ensure incorporation of vesicle contents into the cells. Biochim Biophys Acta 509:272, 1978.
29. LESERMAN LD, WEINSTEIN JN, BLUMENTHAL R, ET AL. Receptor-mediated endocytosis of antibody opsonized liposomes by tumor cells. Proc Natl Acad Sci USA 77:4089, 1980.
30. LESERMAN LD, BARBET J, KOURILSKY FM, ET AL. Targeting to cells of fluorescent liposomes covalently coupled with monoclonal antibody or protein A. Nature 288:602, 1980.
31. MARTIN FJ, and PAPAHADJOPOULOS D. Irreversible coupling of immunoglobulin fragments to preformed vesicles. J Biol Chem 257:286, 1982.
32. LESERMAN LD, MACHY P, and BARBET J. Cell-specific drug transfer from liposomes bearing monoclonal antibodies. Nature 293:226, 1981.
33. YATVIN MB, WEINSTEIN JN, DENNIS WH, ET AL. Design of liposomes for enhanced local release of drugs by hyperthermia. Science 202:1290, 1978.
34. WEINSTEIN JN, MAGIN RL, YATVIN MB, ET AL. Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. Science 204:188, 1979.
35. WEINSTEIN JN, MAGIN RL, CYSYK RL, ET AL. Treatment of solid L1210 murine tumors with local hyperthermia and temperature-sensitive liposomes containing methotrexate. Cancer Res 40:1388, 1979.
36. YATVIN MB, MUHLENSIEPEN, PORSCHEN, ET AL. Selective delivery of liposome encapsulated *cis*-dichlorodiammineplatinum (II) by heat: influence on tumor drug uptake and growth. Cancer Res 41:1602, 1981.
37. YATVIN MB, KRENTZ W, HORWITZ BA, ET AL. pH-sensitive liposomes: possible clinical implications. Science 210:1253, 1980.
38. ALVING CR, STECK EA, CHAPMAN WL, JR, ET AL. Liposomes in leishmaniasis: therapeutic effects of antimonial drugs, 8-aminoquinolines, and tetracycline. Life Sci 26:2231, 1980.
39. POSTE G, KIRSH R, FOGLER WE, ET AL. Activation of tumoricidal properties in mouse macrophages by lymphokines encapsulated in liposomes. Cancer Res 39:881, 1979.
40. FIDLER IJ, SONE S, FOGLER WE, ET AL. Eradication of spontaneous metastases and activation of alveolar macrophages by intravenous injection of liposomes containing muramyl dipeptide. Proc Natl Acad Sci USA 78:1680, 1981.
41. KLEINERMAN ES, ERICKSON KL, SCHROIT AJ, ET AL. Activation of tumoricidal properties in human blood monocytes by liposomes containing lipophilic muramyl tripeptide. Cancer Res 43:2010, 1983.
42. RYMAN BE, and BARRAT GM. Possible improvement of radioimmunodetection of tumours by liposomal clearance of antibodies to tumour products. Prog Clin Biol Res 102(Part A):299, 1982.
43. ALVING CR, and RICHARD RL. Immunological aspects of liposomes. *In* The Liposomes (Ostro M, ed). New York, Marcel Dekker, 1983.
44. MAYHEW E, and PAPAHADJOPOULOS D. Therapeutic applications of liposomes. *In* The Liposomes (Ostro M, ed). New York, Marcel Dekker, 1983.
45. RICHARDSON VJ, RYMAN BE, JEWKES RF, ET AL. ^{99m}Tc-labelled liposomes: preparation of radiopharmaceutical and its distribution in a hepatoma patient. Int J Nucl Biol Med 5:118, 1978.
46. RICHARDSON VJ, RYMAN BE, JEWKES RF, ET AL. Tissue distribution and tumor localization of technetium-99M labeled liposomes in cancer patients. Br J Cancer 40:35-43, 1979.
47. RICHARDSON VJ, RYMAN BE, JEWKES RF, ET AL. ^{99m}Tc-labelled liposomes and mammary carcinoma.
48. GREGORIADIS G, SWAIN P, WILLS EJ, ET AL. Drug-carrier potential of liposomes in cancer chemotherapy. Lancet i:1313, 1974.

49. SEGAL AW, GREGORIADIS G, LAVENDER JP, ET AL. Tissue and hepatic subcellular distribution of liposomes containing bleomycin after intravenous administration to patients with neoplasms. Clin Sci Mol Med 51:421, 1976.

Antitumor Antibiotics and Low Molecular Weight Immunomodifiers of Microbial Origin

Hamao Umezawa[1,*]

Antitumor antibiotics research has made great progress both in the development of new types of antitumor cytotoxic compounds with potential usefulness and in the development of effective derivatives and analogs of already proven compounds such as doxorubicin, bleomycin, and mitomycin. Such research has also been expanded to include the study of low molecular weight enzyme inhibitors and immunomodifiers of microbial origin, which, with their derivatives and analogs, can also be expected to increase the cure rate for cancer. Recent results of research to discover and learn about these compounds are described here, after a brief historical introduction. [Cancer Treat Rep 68:137-144, 1984]

The discovery of the therapeutic effect of penicillin by Florey et al of the University of Oxford began the first era of antibiotics research. By 1951, penicillin, streptomycin, chloramphenicol, chlortetracycline, oxytetracycline, and erythromycin had been introduced into clinical use, and chemotherapy for bacterial and rickettsial diseases had been almost completely established. It can be said that, thereafter, antibiotics research entered its second era, where the derivatives and analogs of such compounds as penicillin and aminoglycoside antibiotics were developed for the treatment of infections by resistant organisms and where investigation was no longer limited to antibacterial compounds.

In 1951, my collaborators and I initiated screening for antitumor substances produced by micro-organisms; in 1953, we issued the first report of the discovery of two microbial products, substance 289 and sarkomycin (1) (this was the first report of the successful screening for antitumor antibiotics). In 1954, azaserine was reported by Ehrlich et al, and this expansion in antibiotics research gave rise to the term "antitumor antibiotics," defined as compounds that are produced by micro-organisms and inhibit the growth of tumor cells and tumors.

By 1965, nuclear magnetic resonance and x-ray crystal analysis had been introduced into natural product chemistry, and it became possible to elucidate the structures of low molecular weight microbial products very quickly. Moreover, at about that same time, rapid progress began towards an understanding of the biochemistry of various diseases. With the aim of exploiting a new research area of microbial products, I initiated screening for low molecular weight enzyme inhibitors. In this study (until 1972), we discovered, for example, specific inhibitors of various proteases (2) and inhibitors of enzymes involved in the biosynthesis of noradrenaline (3). Substances thus found included: leupeptin (inhibiting plasmin, trypsin, papain, etc); antipain (inhibiting trypsin, papain, etc); chymostatin (inhibiting chymotrypsin strongly and papain weakly); elastatinal (inhibiting papain); pepstatin (inhibiting pepsin, cathepsin D, and renin); and phosphoramidon (inhibiting metalloproteinases). They have been widely used for the biochemical analysis of biologic functions and disease processes or for the extraction and purification of important peptides and proteins to protect them from proteolytic degradation during their extraction. They have also been used as the functional group in affinity chromatography; for instance, renin was, for the first time, purified by affinity chromatography using pepstatin as the functional group. All inhibitors of tyrosine hydroxylase, dopa-decarboxylase, and dopamine β-hydroxylase had hypotensive activity. As described in a recent review (3), we have so far discovered about 50 inhibitors of various enzymes and have elucidated their chemical structures. The study of low molecular weight enzyme inhibitors will be continued and will contribute to the development of new therapeutic agents. Moreover, the discovery of enzyme inhibitors and thousands of antibiotics indicates that micro-organisms produce an almost unlimited number of various

---

[1] Microbial Chemistry Research Foundation, Institute of Microbial Chemistry, Tokyo, Japan.

[*] *Reprint requests to:* Hamao Umezawa, MD, Microbial Chemistry Research Foundation, Institute of Microbial Chemistry, 14-23 Kamiosaki 3-Chome, Shinagawa-ku, Tokyo 141, Japan.

compounds that have various activities. It indicates that if a quantitatively exact screening method is applied, then the sought-after compounds are very likely to be found. I initiated the screening study for low molecular weight immunomodifiers and in 1976 reported the discovery of interesting immunity-enhancing agents; one of them, "bestatin," has been studied clinically. During the period of these studies on immunomodifiers, cancer immunology was making great progress, and suppressor cells which strongly suppress immunoresistance were confirmed to be increased in many cancer patients. Thus started the study of microbial products that might suppress the generation or action of suppressor cells.

In the following paragraphs I will review our recent studies on antitumor antibiotics and low molecular weight immunomodifiers including those inhibiting the generation or action of suppressor cells, and I will discuss the possibilities for future progress.

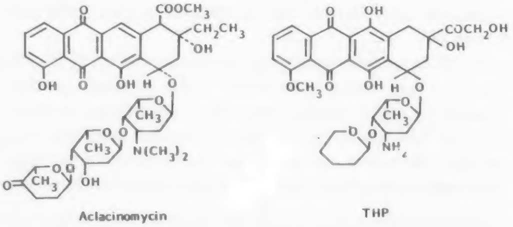
Antitumor Antibiotics, Their Derivatives, and Their Analogs With Potential Usefulness

In 1941, starting the screening for antibacterial antibiotics, Waksman et al were successful in isolating an antibacterial antibiotic as deep-red crystals from culture filtrates of *Actinomyces antibioticus* and named it "actinomycin"; in 1961 Farber used this antibiotic in patients with Wilms' tumor and established a very effective method of treatment.

In the late 1950s, Brockmann was interested in the chemistry of the other group of red-pigment antibacterial antibiotics; he isolated them, determined their structures, and named them "anthracyclines." The aglycone of anthracycline, that is, the main structural moiety without the sugar moiety, was named "anthracyclinone." Daunorubicin, an anthracycline antibiotic discovered by Di Marco and Lenaz (4), was confirmed to be useful in the treatment of leukemia. A derivative of daunorubicin, ie, doxorubicin, was discovered by Arcamone (5) and was found by clinical study to have a strong effect in destroying, for example, leukemia cells, lymphoma, breast cancer, and ovarian tumors. However, the total dose of doxorubicin was limited by its cardiac toxicity, and it was imagined that stronger therapeutic effect would be achieved by finding an analog or derivative of doxorubicin that had lower cardiac toxicity. Thus, in the last 10 years many researchers have undertaken the study of new anthracyclines. Arcamone himself has synthesized a great many derivatives of daunorubicin and doxorubicin. Among them, 4-epi-doxorubicin and 4-demethoxydaunorubicin have been studied clinically. The latter can be given orally to patients and exhibits therapeutic effects.

My collaborators and I also believed in the importance of developing new anthracyclines for the treatment of cancer. In our study (6), we discovered aclarubicin (fig 1). It had stronger activity than daunorubicin but weaker activity than doxorubicin in inhibiting L1210 mouse leukemia. Dantchev et al (7) found that aclarubicin had significantly lower cardiac toxicity than doxorubicin in hamsters. Clinical study of aclarubicin has confirmed its low cardiac toxicity and its usefulness in the treatment of leukemia.

We discovered another group of new anthracyclines, which we named baumycins A1, A2, B1, and B2. A1 showed a strong inhibitory effect on L1210 leukemia, but this was not repeatedly observed in other laboratories. Baumycins had the structures of 4′-*O*-acetal derivatives of daunorubicin. Therefore, we synthesized 4′-*O*-acetal and hemiacetal derivatives of doxorubicin and daunorubicin. Among 50 derivatives thus synthesized and compared with doxorubicin, only one was found to have the same or stronger activity against L1210 leukemia. This had the structure of 4′-*O*-tetrahydropyranyldoxorubicin (fig 1). Depending on the configuration of the 4′-carbon atom, there are two isomers of the compounds shown by this structure. We synthesized both of them and found that one of them had antitumor activity that equaled or exceeded that of doxorubicin (8). This active compound, theprubicin (THP), was confirmed by Dantchev et al to have low cardiac toxicity in hamsters; the cardiac toxicity of THP was lower than that of doxorubicin. In clinical studies of THP, side effects such as alopecia, nausea, and vomiting occurred much less frequently than in trials with doxorubicin. THP has shown a strong therapeutic effect in the treatment of lymphoma, lymphatic leukemia, and tumors of the ovary, uterus, and head and neck. It was taken up very rapidly by mouse tumors, which are inhibited by this compound; by 2 minutes after the addition of THP to cultures of mouse tumor cells such as L5178Y or P388, the intracellular level of THP has already reached a value close to maximum uptake. Uptake velocity for THP was about 170 times faster than that for doxorubicin (9). When 10^7 cells were cultured for 10 minutes in 5 ml of a culture medium containing THP at 10 μg/ml, 27 μg was taken up by the cells and 73% of the THP thus taken up was located in nuclei. This value, that is, 27 μg/10^7 cells, suggests that the intracellular concentration of THP was 2.7 mg/ml. This is surprisingly high. THP inhibited DNA synthesis, and the degree of this activity was almost the same as that of doxorubicin. These findings suggest that tumors which are very sensitive to



Aclacinomycin THP

FIGURE 1.—Aclarubicin (aclacinomycin) and 4′-*O*-tetrahydropyranyldoxorubicin (THP).

THP, even large tumors, may be destroyed rapidly and that degradation products of nucleic acids of tumor cells may cause strong renal damage. This has been observed in some lymphoma patients treated with THP; uric acid was increased in blood and caused renal damage.

At present, the mechanism of resistance of cancer cells to compounds such as doxorubicin and the vinca alkaloids is being studied. The efflux of antitumor agents from resistant tumor cells is generally quicker than that from sensitive cells. The efflux of THP from mouse tumor cells that are inhibited by THP was slower than that of doxorubicin. Two P388 cell lines which were resistant to doxorubicin were also resistant to THP. However, the degree of resistance to THP was lower than that to doxorubicin: 50% inhibitory concentration of doxorubicin against two resistant cell lines was 70 times higher than that against the parental sensitive cells, and 50% inhibitory concentration of THP against resistant cell lines was 13 times higher than that against the parental sensitive cells. Thus, the efflux of THP from the resistant cell line was faster than that from their parental cells, but the efflux of THP was slower than that of doxorubicin.

The study of anthracyclines should lead to the discovery of derivatives or analogs with lower toxicity than doxorubicin but with at least equal therapeutic effect. It should also lead to the discovery of new anthracyclines that have no cross-resistance relationships with doxorubicin. Such anthracyclines may be effective in the treatment of pulmonary and gastric cancers, which are resistant to treatment with the anticancer drugs presently available.

In 1957, we discovered the unique antitumor antibiotic phleomycin. It showed a strong effect against Ehrlich carcinoma, and its therapeutic index was much higher than that of other antitumor agents at that time. I planned its clinical study but had to abandon the plan, because phleomycin was found to have strong renal toxicity in dogs. We continued screening for antibiotics of a similar type and discovered a new one which was differentiated from phleomycin by paper chromatography and stability in acid. The new antibiotic was more stable than phleomycin; moreover, it did not have the strong renal toxicity in dogs. We named this antibiotic "bleomycin" and reported it in 1966. Since about 1970, bleomycin has been used in the treatment of Hodgkin's lymphoma and tumors of the skin, head, neck, and testis.

The bleomycin molecule consists of a complicated peptide and a disaccharide containing a carbamoyl group (fig 2). The amino acid, which is located at the left terminal of the peptide moiety of the bleomycin molecule shown in figure 2, contains a pyrimidine ring, and we named this amino acid "pyrimidoblamic acid." In 1972, studying hydrolysis conditions for the successive cleavage of the peptide bonds and isolating the disaccharide moiety, we could propose the structure of bleomycin molecule except for the side chain part of the pyrimidoblamyl moiety. The structure shown in figure 2 was finally determined in 1978 (10). All carbon atoms were identified by ^{13}C-nuclear magnetic resonance. After we reported this structure, all nitrogen atoms in the bleomycin molecule were identified by ^{15}N-nuclear magnetic resonance. We were successful in the synthesis of pyrimidoblamic acid in 1980 (11) and the synthesis of the peptide moiety (deglycobleomycin A2) in 1981. Finally, we were successful in the total synthesis of bleomycin (1981) (12,13), and the structure shown in figure 2 was thus proved conclusively. After our report, others also reported the total synthesis of bleomycin (14). On the basis of this structure, the mechanism of action was elucidated, and the research area for the development of new bleomycin derivatives and analogs was opened.

Bleomycin binds with Cu^{2+} and produces an equimolar complex. We determined the structure of the copper complex (15) and proposed the structure of bleomycin-Fe^{2+}

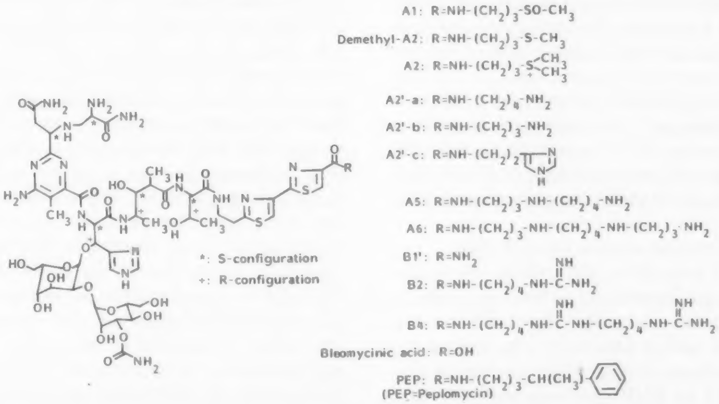

*: S-configuration
+: R-configuration
A1: R=NH-(CH2)3-SO-CH3
Demethyl-A2: R=NH-(CH2)3-S-CH3
A2: R=NH-(CH2)3-S+(CH3)(CH3)
A2'-a: R=NH-(CH2)4-NH2
A2'-b: R=NH-(CH2)3-NH2
A2'-c: R=NH-(CH2)2-
A5: R=NH-(CH2)3-NH-(CH2)4-NH2
A6: R=NH-(CH2)3-NH-(CH2)4-NH-(CH2)3-NH2
B1': R=NH2
B2: R=NH-(CH2)4-NH-C(=NH)-NH2
B4: R=NH-(CH2)4-NH-C(=NH)-NH-(CH2)4-NH-C(=NH)-NH2
Bleomycinic acid: R=OH
PEP: R=NH-(CH2)3-CH(CH3)-
(PEP=Peplomycin)

FIGURE 2.—Bleomycins and peplomycin.

complex (15). Bleomycin hydrolase, which is widely distributed among human and animal cells, hydrolyzes the amide bond of the α-aminocarboxamide part of the pyrimidoblamyl moiety of bleomycin. Bleomycin copper complex is resistant to this enzyme. Bleomycin, injected into animals, binds to Cu^{2+} in the blood, and, after penetrating into the cells, the Cu^{2+} of the bleomycin-copper complex is reduced; the Cu^{+} thus formed is transferred to a cellular protein that can bind to Cu^{+}. Copper-free bleomycin thus formed is subject to the action of bleomycin hydrolase and is inactivated. Bleomycin that escapes this enzyme reaction goes to nuclei and reacts with DNA (16).

Bleomycin binds to DNA, the bithiazole moiety intercalates between bases of double-stranded DNA, and the terminal amine is also involved in the binding.

The bleomycin-Fe^{2+}-O$_2$ complex binds to DNA and causes double-strand scission. The active form of the complex involved in the reaction causing DNA fragmentation has been suggested to be bleomycin-Fe^{3+}-O$_2^{2-}$ (16). Bleomycin-Fe^{3+}-O$_2^{2-}$, which binds to DNA, abstracts the hydrogen radical from the 4-C of the 2-deoxyribose moiety of DNA and this is followed by the reactions resulting in DNA strand scission (16).

We have established both fermentation and chemical methods for the preparation of artificial bleomycins that contain various terminal amines. Thus, we have prepared > 400 various bleomycins, and we have found that the degree of renal and pulmonary toxicity is different among these bleomycins. Peplomycin (fig 2), one of the artificial bleomycins, has been studied clinically. It was confirmed to have a therapeutic effect not only against tumors sensitive to bleomycin treatment but also against prostatic carcinoma; it also has lower pulmonary toxicity than bleomycin. We are developing other bleomycin analogs which have lower toxicity than peplomycin.

Radioactive metal complexes of bleomycin are taken up by tumors, especially by lung cancer, and are useful for diagnosis. Therefore, we assumed that the bleomycin analogs and derivatives resistant to bleomycin hydrolase would have a wider anticancer spectrum than bleomycin. Modification of the α-aminocarboxamide part of the bleomycin molecule has given such derivatives.

We further improved (17) the method used in the total synthesis of bleomycin, and, using this method, we have synthesized three analogs which have given useful information on structure-activity relationships. It is certain that some new analogs will be developed by chemical synthesis.

Derivatives of mitomycin are also being studied.

Besides studies of derivatives and analogs, the screening for new antitumor antibiotics has been continued by applying various other methods. The antibiotic bactobolin was found in culture filtrates of a bacterial strain classified as *Pseudomonas* (18). Most antitumor compounds inhibit DNA or RNA synthesis and have bone marrow toxicity, but bactobolin (fig 3) inhibits protein synthesis on ribosomes. Most cancer chemotherapeutic agents except for bleomycin have bone marrow toxicity and are given to patients every 3 to 4 weeks. An antibiotic such as bactobolin may be given during the period when administration of chemotherapeutic agents which have bone marrow toxicity is interrupted. In this respect, glyo II (19) (fig 3), which is produced by streptomyces and inhibits glyoxalase II, is interesting, because the growth of mouse tumors is suppressed during the period of administration of this antibiotic. Among pyrrolo(1,4)benzodiazepine antibiotics, neothramycin (20) (fig 3) has the lowest toxicity. It has a strong therapeutic effect against rat hepatomas. Therefore, neothramycin has been studied clinically. Shrinkage of tumors was very slight, but the enlargement of tumors seemed to be suppressed during the administration of neothramycin.

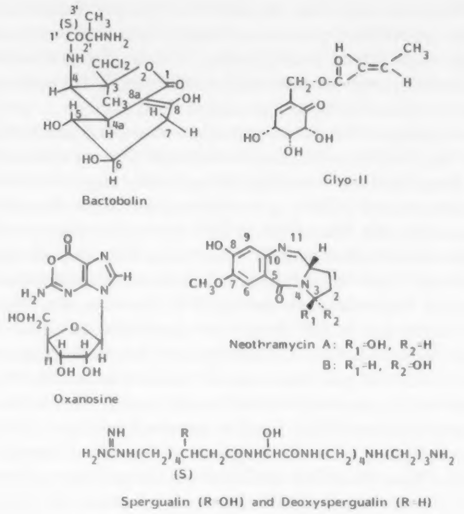

Bactobolin
Glyo-II
Oxanosine
Neothramycin A: R1=OH, R2=H
B: R1=H, R2=OH
H2NCNH(CH2)4CHCH2CONHCHCONH(CH2)4NH(CH2)3NH2
Spergualin (R=OH) and Deoxyspergualin (R=H)

FIGURE 3.—Antitumor antibiotics with potential usefulness.

In 1981, we discovered an antitumor antibiotic which has a completely new type of structure (21); it was produced by *Bacillus laterosporus* and its molecule was found to contain the spermidine moiety and a guanidine group. Therefore, we named this antibiotic "spergualin" (fig 3). Spergualin has a strong inhibitory effect on L1210 mouse leukemia. We synthesized its derivatives and found that 15-deoxyspergualin has a stronger antitumor activity than spergualin. The antitumor action of spergualin is not due to the inhibition of synthesis of DNA, RNA, or protein. The mechanism of its action is interesting, and it is expected that spergualin or its analog will exhibit therapeutic effect on, for example, leukemia and lymphoma. Clazamycin (22) was recently found to interfere with cell membranes and to exhibit antitumor effects. It should be interesting to test the clinical effect of antitumor compounds interfering with membrane functions on gastric and pulmonary cancers, which are resistant to the presently available chemotherapy. Oxanosine (fig 3) is a nucleoside antibiotic of a new structural type. As described later, this antitumor antibiotic is becoming interesting in its effect on suppressor cells (16).

Low Molecular Weight Immunomodifiers Produced by Micro-organisms

In 1972, testing the effect of antitumor antibiotics on immune responses, we found that the administration of a very small dose of diketocoriolin B (fig 4) to mice increased the number of spleen cells producing antibody (23). Coriolins (fig 4) are antitumor antibiotics which we discovered in culture filtrates of a mushroom classified as *Coriolus consor.* This mushroom is called "saruno-koshikake" in Japanese, and its crude extract has been used as a folk medicine for cancer patients. Diketocoriolin B is a derivative of coriolin B. A very low dose of coriolins and diketocoriolin B increased the number of antibody-forming cells in mouse spleen. We also found that coriolins and diketocoriolin B inhibit Na^+-K^+-ATPase. This enzyme is located in cell membranes of all kinds of animal cells. I thought, therefore, that the binding of diketocoriolin B to membranes of the cells involved in antibody formation resulted in the increased number of antibody-forming cells. Later we found that diketocoriolin B acts on the antibody-forming B lymphocytes. I assumed that the screening for compounds that bind to cell membranes or surfaces would result in the discovery of immunity-modifying compounds (24). Inhibitors of enzymes located on cell surfaces or membranes should bind to cells. Therefore, to find microbial products that could bind to cells, we first studied the enzymes on the cell surfaces and searched for their inhibitors. In this study we found that all aminopeptidases that hydrolyze *N*-terminal peptide bonds are located on the cell surface. These enzymes are not released extracellularly. We also found that alkaline phosphatases and esterases are located on the cell surfaces. Searching for inhibitors of these enzymes, we discovered bestatin [inhibiting aminopeptidase B (Ki = 6×10^{-8} M) and leucine aminopeptidase (Ki = 2×10^{-8} M)], amastatin [inhibiting aminopeptidase A (Ki = 1.5×10^{-7} M) and leucine aminopeptidase (Ki = 1.5×10^{-6} M)], forphenicine [inhibiting chicken intestine alkaline phosphatase], esterastin (Ki = 2×10^{-10} M) and ebelactones A and B [inhibiting esterase (Ki = 9.2×10^{-8} M for A; Ki = 5×10^{-10} M for B)]. We determined their structures as shown in figure 5 (24). All these inhibitors have a very low toxicity.

Very recently, we have found two inhibitors that are completely specific for aminopeptidase B. We elucidated their structure (fig 6) and named them "arphamenines." These inhibitors were isolated from culture filtrates of a bacterial strain classified as *Chromobacterium violaceum.* Arphamenines A and B inhibit aminopeptidase B very strongly; 50% inhibition concentrations are 0.0006 μg/ml for A (Ki = 2.5×10^{-9} M) and 0.002 μg/ml for B (Ki = 8×10^{-10} M). However, they do not inhibit aminopeptidase A or leucine aminopeptidase.

At low doses (1–100 μg/mouse), bestatin enhanced delayed-type hypersensitivity to sheep rbcs, and at higher doses (1 mg/mouse) it increased the number of antibody-forming cells in the spleen. Amastatin increased the number of antibody-forming cells. Forphenicine (1–100 μg/mouse) enhanced delayed-type hypersensitivity and also increased the number of antibody-forming cells when given at 10–1000 μg/mouse. Ebelactone A enhanced delayed-type hypersensitivity, but esterastin suppressed delayed-type hypersensitivity and reduced the number of antibody-forming cells. Arphamenine A enhanced delayed-type hypersensitivity (16,24).

The type of inhibition of chicken intestine alkaline phosphatase by forphenicine was not competitive with the substrate. Its derivative, forphenicinol, which contains a hydroxymethyl group instead of the aldehyde group in the forphenicine molecule, did not inhibit alkaline phosphatase but did bind to cells. Forphenicinol enhanced delayed-type hypersensitivity.

Among these low molecular weight immunomodifiers, bestatin, forphenicine, forphenicinol, and arphamenines A and B have been tested for antitumor effects against mouse tumors; all showed antitumor action against some mouse tumor strains in vivo. They have no cytotoxicity.

Of these immunomodifiers, bestatin has been studied in greatest detail (24). Experimental results from testing the effects of bestatin on the mouse immune system can be summarized as follows.

(*a*) At 1, 10, or 100 μg/mouse given orally at the time of immunization, bestatin enhanced delayed-type hypersensitivity to sheep rbcs.

(*b*) At 1000 μg/mouse, bestatin did not enhance delayed-type hypersensitivity but did increase the number

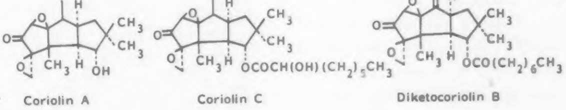


FIGURE 4.—Coriolins and diketocoriolin B.

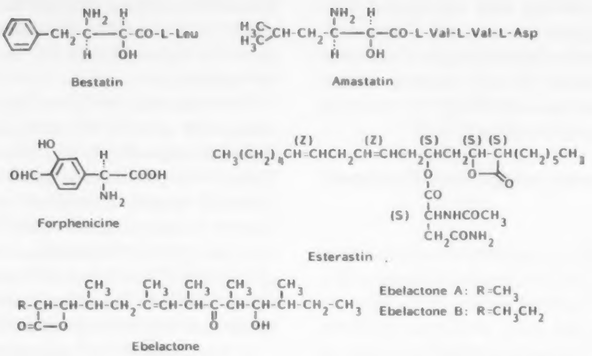


FIGURE 5.—Low molecular weight immunomodifiers produced by micro-organisms.

of mouse spleen cells producing antibody to sheep rbcs.

(*c*) Delayed-type hypersensitivity against oxazolone, which had been reduced by cyclophosphamide or by ip inoculation of Ehrlich carcinoma cells, was restored to the normal level by oral administration of 10-1000 μg of bestatin.

(*d*) Delayed-type hypersensitivity to sheep rbcs, which had been reduced by inoculation of Ehrlich carcinoma cells, was enhanced by bestatin (10 μg/mouse).

(*e*) Bestatin (0.001-0.1 μg/ml) stimulated the differentiation of bone marrow stem cells into granulocytes in a medium containing colony-stimulating factor. This effect was also observed in vivo.

(*f*) Bestatin (1, 10, or 100 μg daily) showed inhibitory effects against solid tumors, eg, Gardner lymphosarcoma and IMC carcinoma.

(*g*) When P388 leukemia cells were inoculated into the footpad, bestatin suppressed its metastasis into the axial lymph nodes.

(*h*) Bestatin enhanced the effect of clinically useful antitumor agents such as bleomycin, doxorubicin, aclarubicin, and mitomycin.

(*i*) Bestatin retarded the induction of skin cancer by methylcholanthrene.

(*j*) Bestatin suppressed the occurrence of spontaneous tumors in aged mice.

(*k*) Bestatin (10 μg/mouse) prevented bacterial infections in mice treated with cyclophosphamide.

(*l*) Oral administration of > 10 μg of bestatin/mouse increased [^{3}H]thymidine incorporation into spleen cells 24 hours after its administration.

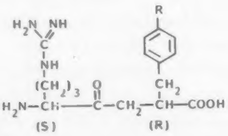


FIGURE 6.—Arphamenines A (R=H) and B (R=OH).

(*m*) Bestatin stimulated the production of interleukins 1 and 2.

As reported by Müller et al, the administration of bestatin to mice induces an increase in [^{3}H]thymidine incorporation into DNA of T cells but not B cells. An increased activity of DNA polymerase α was observed in T cells but not in B cells, whereas activity of DNA polymerase β was not influenced by bestatin administration. It has also been found that deoxynucleotidyl terminal transferase activity in bone marrow is increased by bestatin administration (16,24).

Bestatin has been studied clinically during the last 5 years. Its effects on the human immune system may be summarized as follows.

(*a*) Daily doses of 30 or 60 mg increased the percentage and number of T cells that had been reduced in cancer patients.

(*b*) The reduced NK cell activity in cancer patients was restored.

(*c*) The action of cancer patient serum in inhibiting IgG formation by peripheral leukocytes was eliminated by bestatin both in vitro and in vivo.

(*d*) Bestatin increased the number of nucleated cells in the bone marrow of cancer patients.

(*e*) After bestatin treatment, negative skin reactions in anergic patients became positive in high frequency.

Clinical randomization tests for therapeutic effects of bestatin are being done in patients with melanoma, bladder cancer, tumors of the head and neck, carcinoma of the esophagus and of the stomach, and leukemia. The effects of bestatin in inhibiting recurrences and prolonging the survival period or in increasing the rate of complete cure are being confirmed; for instance, the survival period of patients with melanoma after macroscopic elimination of tumors by surgery and chemotherapy has been significantly prolonged by bestatin administration (30 mg daily, orally) compared with the control group not receiving bestatin treatment.

In the usual animal experiments testing for the effect of antitumor compounds in inhibiting the growth of tumors, first tumor cells are inoculated, on the following day the daily administration of antitumor compounds is started, and this administration is continued for 7 or 10 days. The additional treatment with bestatin or other immunity-enhancing agents augments the effect of antitumor compounds and the number of surviving mice increases. Moreover, mice that survive gain immunity to the tumor and reject the second inoculation of cells from the same tumor. However, it is thought that to achieve a similar synergistic effect of immunity-enhancing compounds with antitumor compounds in the treatment of human cancer, it may be necessary to inhibit the generation or action of suppressor cells, because the suppressor cells that suppress immunity have increased in cancer patients. Therefore, we have initiated the study of microbial products that might inhibit the generation of suppressor cells.

It is reasonable to assume that there exist cytotoxic antibiotics that inhibit the generation of suppressor cells more strongly than that of other immune cells. In fact, anticancer drugs such as cyclophosphamide, 6-mercaptopurine, and aclarubicin have been confirmed to inhibit the generation of suppressor cells in mice.

We found that oxanosine (25) inhibits both the generation and action of suppressor cells that suppress delayed-type hypersensitivity to sheep rbcs. Iv injection of a large number of sheep rbcs (eg, 10^8) induces the generation of suppressor cells that inhibit the induction of delayed-type hypersensitivity to sheep rbcs. Oxanosine (100-400 mg/kg), given ip 2, 3, and 4 days (ie, three times) after iv injection of 10^8 sheep rbcs, showed from 70% to 100% inhibition of suppressor cell generation. Encouraged by this observation, we are studying derivatives and analogs of oxanosine. Although oxanosine is an antitumor antibiotic, it has low toxicity.

It is also possible to find compounds inhibiting the generation of suppressor cells among low molecular weight immunomodifiers. We have confirmed that bestatin, forphenicinol, and arphamenines A and B inhibit the generation of suppressor cells. These compounds have no cytotoxic action. The effects of arphamenines A and B inhibiting mouse tumors are suggested to be due to their action in inhibiting the generation and action of suppressor cells.

We have not yet been successful in establishing a useful method for testing the effect of immunomodifiers on suppressor cells that suppress the resistance to cancer. We found that the administration of cyclophosphamide to animals 1 or 3 days after the inoculation of IMC carcinoma cells does not inhibit growth of the tumor; however, administration 6 days after inoculation of tumor cells inhibits tumor growth. This antitumor effect produced by one or two injections 6 days after tumor cell inoculation may be due to inhibition of the generation or action of suppressor cells that reduce or eliminate the resistance to cancer. In this test, oxanosine, which has low toxicity, produced an effect similar to that of cyclophosphamide. Bestatin, forphenicine, forphenicinol, and arphamenines also produced this effect.

Compounds inhibiting the generation or action of suppressor cells should enhance the effect of chemotherapy in eliminating tumors macroscopically. If the generation or action of suppressor cells is inhibited, then low molecular weight immunity-enhancing agents, such as bestatin, etc, that have very low toxicity should augment the effect of chemotherapy. The residual tumors that remain after macroscopic elimination can be eliminated by administration of immunity-enhancing agents which restore the reduced immunity in cancer patients, or by additional chemotherapy. Use of the proper combination of different immunity-enhancing agents such as bestatin, forphenicine, forphenicinol, and arphamenine may produce a stronger therapeutic effect than any one of them used alone.

CONCLUSION

The study of derivatives and analogs of antitumor antibiotics which have been used for cancer treatment has led to the development of chemotherapeutic agents which are more effective than their parental compounds. It is also leading to the development of agents which are effective against the types of cancer that are resistant to treatment with the parental compounds. Screening methods have been modified or new methods have been devised, and interesting new antitumor antibiotics have been found and selected for clinical study. It is almost certain that screening for activity inhibiting L1210 mouse leukemia and Ehrlich carcinoma has led to the discovery of antitumor compounds that are useful in the treatment of such cancers as leukemia, lymphoma, and tumors of the ovary, testis, skin, head, and neck; however, it has not yet led to the discovery of compounds which can produce a therapeutic effect against gastric or pulmonary cancer. It may be interesting to subject antitumor compounds to tests on resistant tumors, even though the compounds have no or weak activity against L1210. Screening for compounds that are effective against resistant cell lines will lead to the discovery of interesting antitumor agents.

The study of immunomodifiers has again demonstrated that micro-organisms produce an unlimited number of secondary metabolites which have various structures with various kinds of pharmacologic activities.

Many cancer researchers are not very interested in immunity-enhancing agents. They should do some experiments with immunomodifiers, in a clean experimental room, using mice that have been kept in sanitary conditions (infection in mice disturbs the results of the experiment). If they test the effect of an immunity-enhancing agent in combination with antitumor cytotoxic com-

pounds, they will be impressed by the marked increase in chemotherapeutic effect.

REFERENCES

1. UMEZAWA H, TAKEUCHI T, NITTA K, ET AL. Studies on anti-tumor substances produced by microorganisms. III. On sarkomycin produced by a strain resembling *Streptomyces erythrochromogenes*. J Antibiot A6:147-152, 1953.
2. UMEZAWA H. Structures and activities of protease inhibitors of microbial origin. Methods Enzymol 45:678-695, 1976.
3. _____. Low molecular weight enzyme inhibitors of microbial origin. Annu Rev Microbiol 36:75-99, 1982.
4. DI MARCO A, and LENAZ L. Daunomycin and adriamycin. *In* Cancer Medicine (Holland JF, and Frei E, eds). Philadelphia, Lea and Febiger, 1973, pp 826-835.
5. ARCAMONE F. Doxorubicin—Anticancer Antibiotics. New York, Academic Press, 1981.
6. OKI T, MATSUZAWA Y, YOSHIMOTO A, ET AL. New antibiotics, aclacinomycins A and B. J Antibiot 28:830-834, 1975.
7. DANTCHEV D, PAINTRAND M, HAYAT M, ET AL. Low heart and skin toxicity of a tetrahydropyranyl derivative of adriamycin (THP-ADM) as observed by electron and light microscopy. J Antibiot 32:1085-1086, 1979.
8. UMEZAWA H, TAKAHASHI T, KINOSHITA M, ET AL. Tetrahydropyranyl derivatives of daunomycin and adriamycin. J Antibiot 32:1082-1084, 1979.
9. KUNIMOTO S, MIURA K, TAKAHASKI Y, ET AL. Rapid uptake by cultured tumor cells and intracellular behavior of 4′-*O*-tetrahydropyranyladriamycin. J Antibiot 36:312-317, 1983.
10. TAKITA T, MURAOKA Y, NAKATANI T, ET AL. Chemistry of bleomycin. XIX. Revised structures of bleomycin and phleomycin. J Antibiot 31:801-804, 1978.
11. UMEZAWA Y, MORISHIMA H, SAITO S, ET AL. Synthesis of the pyrimidine moiety of bleomycin. J Am Chem Soc 102:6630-6631, 1980.
12. TAKITA T, UMEZAWA Y, SAITO S, ET AL. Total synthesis of bleomycin. Peptides: synthesis-structure-function. *In* Proceedings of the 7th American Peptide Symposium (Rich DH, and Gross E, eds). Pierce Chemical Co, 1981, pp 29-39.
13. TAKITA T, UMEZAWA Y, SAITO S, ET AL. Total synthesis of bleomycin A2. Tetrahedron Lett 23:521-524, 1982.
14. AOYAGI Y, KATANO K, SUGUNA H, ET AL. Total synthesis of bleomycin. J Am Chem Soc 104:5537-5538, 1982.
15. TAKITA T, MURAOKA Y, NAKATANI T, ET AL. Chemistry of bleomycin. XXI. Metal-complex of bleomycin and its implication for the mechanism of bleomycin action. J Antibiot 31:1073-1077, 1978.
16. UMEZAWA H. Studies of microbial products in rising to the challenge of curing cancer. (The Leeuwenhoek Lecture, 1982). Proc R Soc Lond [Biol] B217:357-376, 1983.
17. SAITO S, UMEZAWA Y, YOSHIOKA T, ET AL. An improved total synthesis of bleomycin. J Antibiot 36:92-95, 1983.
18. KONDO S, HORIUCHI Y, HAMADA M, ET AL. A new antitumor antibiotic, bactobolin produced by *Pseudomonas*. J Antibiot 32:1069-1071, 1979.
19. CHIMURA H, NAKAMURA H, TAKITA T, ET AL. The structure of a glyoxalase I inhibitor and its chemical reactivity with SH-compound. J Antibiot 28:743-748, 1975.
20. TAKEUCHI T, MIYAMOTO M, ISHIZUKA M, ET AL. Neothramycins A and B, new antitumor antibiotics. J Antibiot 29:93-96, 1976.
21. UMEZAWA H, KONDO S, IINUMA H, ET AL. Structure of an antitumor antibiotic, spergualin. J Antibiot 34:1622-1624, 1981.
22. HORIUCHI Y, KONDO S, IKEDA T, ET AL. New antibiotics, clazamycins A and B. J Antibiot 32:762-764, 1979.
23. ISHIZUKA M, IINUMA H, TAKEUCHI T, ET AL. Effect of diketocoriolin B on antibody formation. J Antibiot 25:320-321, 1972.
24. UMEZAWA H. Small Molecular Immunomodifiers of Microbial Origin—Fundamental and Clinical Studies of Bestatin. Tokyo, Japan, Scientific Societies Press, Oxford, Pergamon Press, 1981.
25. SHIMADA N, YAGISAWA N, NAGANAWA H, ET AL. Oxanosine, a novel nucleoside from actinomycetes. J Antibiot 34:1216-1218, 1981.

Role of Bone Marrow Transplantation in the Treatment of Hematologic Malignancies and Solid Tumors: Critical Review of Syngeneic, Autologous, and Allogeneic Transplants[1]

Richard E. Champlin* and Robert Peter Gale[2]

Intensive chemoradiotherapy and bone marrow transplantation is effective for selected patients with acute or chronic leukemia, lymphoma, or solid tumors. This paper will summarize the results of treatment utilizing syngeneic transplants from identical twins, allogeneic transplants from HLA identical and nonidentical donors, and the use of the patient's own bone marrow cells in autologous bone marrow transplantation. The potential complications of marrow transplantation are described and the current results of bone marrow transplantation are compared with recent advances in alternative forms of treatment for these malignancies. [Cancer Treat Rep 68:145–161, 1984]

Bone marrow transplantation is being increasingly utilized in the treatment of leukemia, lymphoma, and selected solid tumors. This review will outline the principles of treatment using this approach, the major complications and limitations of bone marrow transplantation, and a comparison of the treatment results with those currently achieved with conventional chemotherapy and irradiation.

Principles of Treatment

Acute leukemia and many solid tumors exhibit a dose-related response to irradiation and a number of chemotherapeutic drugs; an increase in the dose leads to a greater cytotoxic effect (1). The dose of many antineoplastic agents that can be administered clinically is limited by toxicity to normal tissues. Bone marrow suppression is the dose-limiting toxic effect for most of these agents. The doses of irradiation and many drugs can be substantially escalated to more effective levels if followed by transplantation of normal hematopoietic cells, rescuing the patient from severe and prolonged myelosuppression (2-5). Three sources of hematopoietic cells have generally been utilized: syngeneic bone marrow from a genetically identical twin, allogeneic bone marrow cells from an HLA identical sibling donor, or cryopreserved autologous bone marrow cells.

A typical scheme for bone marrow transplantation is shown in figure 1. Patients received pretransplant "conditioning treatment" with high doses of chemotherapy and/or irradiation, generally administered over 4-10 days. This treatment must eradicate the neoplastic cells and in the case of allogeneic transplants must also suppress the recipient's immunity sufficiently to prevent rejection of the marrow transplant. The transplant involves only iv infusion of bone marrow cells collected from the donor by multiple bone marrow aspirations (6). An allogeneic transplant requires approximately 1-3 × 10^8 nucleated bone marrow cells/kg of recipient body weight. Approximately 1-5 × 10^7 marrow cells/kg are sufficient for syngeneic or autologous transplantation (7). A number of these cells ultimately home to the bone marrow, engraft, and lead to recovery of normal hematopoiesis usually within 3-4 weeks. If the donor bone marrow cells are not genetically identical to the recipient, posttransplant immunosuppressive treatment is typically administered with methotrexate or cyclosporine to modify or prevent graft-versus-host disease (GVHD). Following transplantation, cytogenetic and gene marker studies have demonstrated that hematopoietic and lym-

---

[1] Supported by Public Health Service grants CA-23715 (National Cancer Institute) and RR-00865 (Division of Research Resources), National Institutes of Health, Department of Health and Human Services. Dr. Champlin is the recipient of a New Investigator Award from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD. Dr. Gale is a Scholar of the Leukemia Society of America and the Meyerhoff Visiting Scientist at the Weizmann Institute of Science, Rehovot, Israel.

[2] Transplantation Biology Unit and Department of Medicine (Hematology and Oncology), UCLA School of Medicine, Center for the Health Sciences, Los Angeles, CA.

**Reprint requests to:* Richard E. Champlin, MD, Transplantation Biology Unit, Department of Medicine, UCLA School of Medicine, Center for the Health Sciences, Los Angeles, CA 90024.

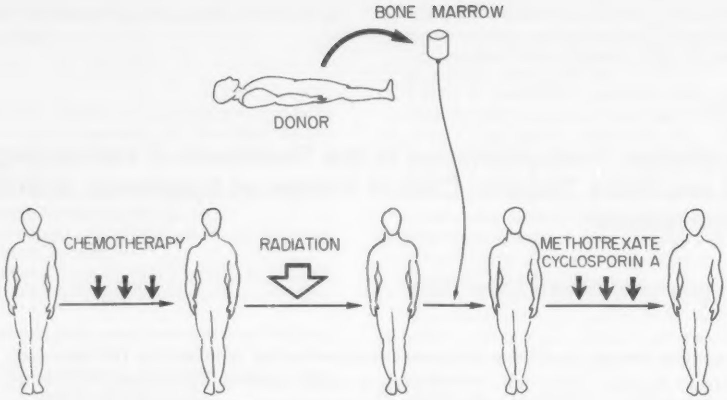


FIGURE 1.—Scheme for allogeneic bone marrow transplantation in the treatment of cancer. The patient typically receives marrow ablative chemotherapy and/or total-body irradiation to eradicate the malignancy and as immunosuppression to prevent transplant rejection. The bone marrow is administered as an iv infusion followed by posttransplant immunosuppression with methotrexate or cyclosporine (cyclosporin A) to prevent GVHD.

phoid tissues are repopulated by progenitors derived from the donor bone marrow (8-11) and that some stromal cells engraft as well (12).

For autologous marrow transplantation, the patient must first undergo collection, cryopreservation, and storage of bone marrow. This should ideally occur at a time when the bone marrow is normally cellular and does not contain malignant cells. At a later time the patient can receive intensive marrow ablative chemotherapy and/or irradiation followed by reinfusion of the cryopreserved marrow to prevent severe myelosuppression (fig 2). With current techniques, marrow cryopreservation can be reliably performed, and the stored cells remain viable for > 5 years (13). The obvious limitation to this approach is the possibility that the marrow may be contaminated by malignant cells at the time of collection. A number of investigators are evaluating techniques to detect microscopic bone marrow involvement and approaches to selectively deplete the marrow of occult leukemia or tumor cells by treatment in vitro with antitumor antibodies, antibody-toxin conjugates, chemotherapy, or physical techniques (14-18).

For treatment with bone marrow transplantation to be successful, the neoplasm must be sufficiently sensitive to chemotherapy and/or irradiation so that one (or possibly several) course(s) of intensive combined modality treat-

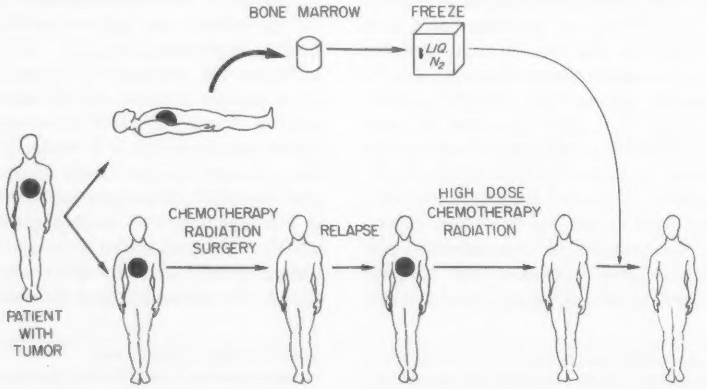


FIGURE 2.—Scheme for autologous bone marrow transplantation. The patient with cancer typically undergoes marrow collection and cryopreservation early in the course of the disease. At a time when the disease is resistant to conventional treatment, intensive combined modality therapy is administered followed by reinfusion of the stored autologous bone marrow cells.

ment may eradicate the malignant cells. Candidate tumors include: acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), lymphoma/Hodgkin's disease, germ cell carcinoma, small cell anaplastic carcinoma of the lung, neuroblastoma, and Ewing's sarcoma. The transplanted bone marrow must contain a sufficient quantity of normal hematopoietic stem cells to allow hematologic recovery within a relatively short time. In the case of autologous transplants the marrow must be devoid of clonogenic malignant cells. In addition, the patients must be in adequate clinical condition to tolerate the intensive antineoplastic therapy.

Potential Complications

Intensive chemoradiotherapy and transplantation of bone marrow may be associated with a number of potential complications, including toxicity of the pretransplant treatment regimen, posttransplant immunodeficiency, infections, interstitial pneumonitis, and in the case of allogeneic transplants, rejection of the transplant and GVHD. The pretransplant conditioning regimens for patients transplanted for leukemia or other malignancies generally include high doses of chemotherapy in combination with total-body irradiation (TBI) (7.5-16 Gy). These regimens approach the limit of tolerance for several tissues. Severe toxicity may occur involving the lung, liver, nervous system, and, rarely, other tissues. Fatal hepatic veno-occlusive disease occurs in approximately 5% of the patients transplanted for leukemia. This is more common in patients with pre-existing liver function abnormalities and in patients who have received extensive previous chemotherapy (19). Interstitial pneumonitis, which may be ascribed to pulmonary toxicity of the conditioning regimen, also occurs. This is often difficult to distinguish from pneumonitis related to cytomegalovirus (CMV) or other viral infections, but probably develops in approximately 5%-10% of patients (20-22). CNS complications are less common but dementia or leukoencephalopathy may occur. Patients with ALL who have had previous CNS involvement or received prophylactic treatment to the nervous system may be at higher risk for this complication (23). Endocrine complications may occur but are generally not life-threatening. Hypothyroidism may develop as a delayed complication 6 months to 2 years after transplant (24). The intensive combined modality therapy typically results in sterility for both males and females, although gonadal endocrine function and ejaculation are usually intact (25). Cataracts are a common delayed complication occurring in most patients who survive > 3 years from transplantation.

The conditioning regimens utilized to treat acute leukemia are sufficiently immunosuppressive that graft rejection from an HLA identical donor is extremely rare. A more important problem is GVHD. Two forms of GVHD have been identified: acute and chronic. Acute GVHD probably results from engraftment of immunocompetent cells from the donor bone marrow which react against recipient tissues. The pathophysiology of acute GVHD is incompletely defined but is probably mediated by T lymphocytes (26). The incidence and clinical manifestations of GVHD are affected by several factors, including the degree of genetic disparity between the donor and recipient, the intensity of the conditioning regimen, and the nature and quantity of the transplanted cells. Primary target organs include skin, liver, and gastrointestinal tract. The clinical and pathologic features have been extensively reviewed (3,6,27,28). Moderate to severe acute GVHD occurs in 30%-60% of allogeneic transplant recipients, typically developing 30-100 days posttransplant, and is fatal in approximately one-half of affected patients. The incidence of acute GVHD increases with recipient age ranging from approximately 10% in patients < 10 years old to ⩾ 70% in patients > 40 years old. For syngeneic and autologous transplants, graft rejection and GVHD should not occur, although a syndrome resembling acute GVHD has been reported in rare patients following transplantation from an identical twin, suggesting that abnormal regulation of immunity may participate in the pathogenesis of that disorder (29).

Several approaches have been proposed to prevent or treat acute GVHD. Based on animal data, most patients have received posttransplant immunosuppression with methotrexate, antithymocyte globulin, cyclosporine, or combinations of agents for 3-6 months (6,30,30a). Patients who have not developed GVHD by this time usually have permanent graft tolerance. Patients who develop GVHD are generally treated with high doses of corticosteroids, antithymocyte globulin, or cyclosporine (31,32). These agents may improve the clinical features of GVHD, but there are no convincing data that survival is improved. An alternative approach to prevent GVHD involves depletion of T lymphocytes from the donor bone marrow in vitro prior to transplantation. A number of techniques have been proposed for this purpose, including E-rosette formation and physical depletion (33), agglutination with lectins (34), treatment with chemotherapy, such as pentostatin or 4-hydroperoxyclyclophosphamide (35), or treatment with anti-T-cell antisera or monoclonal antibodies (36-38). Preliminary clinical trials to evaluate the efficacy of these techniques are in progress.

Chronic GVHD is a separate clinical syndrome affecting approximately 25% of transplant recipients who survive > 6 months (39). Two-thirds of affected patients have preceding acute GVHD but in one-third it develops de novo. The incidence of chronic GVHD also increases with recipient age (40). Chronic GVHD has protean clinical manifestations. Clinical and pathologic features are similar to those seen in several hematologic and autoimmune disorders such as progressive systemic sclerosis, systemic lupus erythematosis, Sjögren's syndrome, and primary biliary cirrhosis (41). Chronic GVHD is a disease of disordered immunity. Patients have a spectrum of immune-related abnormalities, including profound immunodeficiency, autoantibodies, and excessive nonspecific suppressor T-cell activity (42,43). Abnormal immunity is probably the most severe consequence of chronic GVHD; many patients die of infectious complications.

Two forms of chronic GVHD have been described: a limited form with localized skin involvement and an extensive form with generalized skin and multisystem involvement (39). The limited form may not require therapy. The systemic form of chronic GVHD is more serious and is difficult to treat. Corticosteroids, antithymocyte globulin, and other immunosuppressive or cytotoxic agents are usually ineffective in advanced disease. Recently, Sullivan et al (44) reported preliminary encouraging results using a combination of prednisone and either azothioprine or cyclophosphamide in patients with early chronic GVHD. Although this trial was uncontrolled, many patients seemed to benefit and some had regression of their disease.

All patients have a combined immunodeficiency involving both the T- and B-cell axes following marrow transplantation. The most profound abnormalities occur during the first 4-6 months, followed by slow recovery over the next 1-4 years (45-47). Granulocyte and macrophage functions may also be abnormal (48). The intensive pretransplant conditioning treatment utilized to treat leukemia virtually ablates the host immune system. T and B lymphocytes as well as macrophages, monocytes, and other myeloid cells are regenerated from precursor cells present in the donor bone marrow (3). The numbers of circulating T and B cells return to normal levels within 6 weeks but functional studies of these cells are abnormal for at least 1 year. Recipients of syngeneic or autologous transplants also have a period of immunodeficiency but their recovery may be more rapid and posttransplant infections are less severe than in allogeneic transplants (49). Patients with chronic GVHD continue to have profound immunodeficiency for a prolonged period.

Factors thought to be important in determining the severity of posttransplant immunodeficiency in animals and humans include the degree of genetic disparity between donor and recipient, effects of pretransplant radiation and chemotherapy, status of the recipient's thymus, nature of the transplanted lymphoid cells, and the use of posttransplant immunosuppression. Posttransplant immune deficiency is a complex process; several mechanisms are probably operative, including intrinsic or extrinsic blocks in lymphocyte maturation, suppressor cells or factors, and abnormal cell-to-cell interactions between donor lymphocytes and recipient tissues.

Recently, several groups have studied the recovery of T-cell subpopulations in patients recovering from bone marrow transplants using monoclonal antibodies such as the OKT or Leu series. These data indicate a marked decrease in both T helper/inducer ($T_{h/i}$) and suppressor/cytotoxic ($T_{s/c}$) subsets during the immediate posttransplant period. Over the next several months there is a gradual recovery of $T_{s/c}$, with a more delayed recovery of $T_{h/i}$, thus resulting in a reversal of the normal $T_{h/i}$:$T_{s/c}$ ratio. This abnormality appears to precede rather than result from GVHD. Nevertheless, patients with GVHD may exhibit a delayed normalization of T-cell subsets and the ratio may remain abnormal in those with chronic GVHD for prolonged periods (50-52). These abnormalities are not unique to patients receiving bone marrow transplants and are found in individuals with a number of disorders, including the acquired immune deficiency syndrome, collagen-vascular diseases, and in patients recovering from immunosuppressive treatment such as antithymocyte globulin (53,54). Bone marrow transplant recipients also show increased numbers of immature or activated T cells such as those reacting with the OKT-10 and HLA-DR monoclonal antibodies. Most data suggest that these abnormalities are related to cell immaturity rather than activation since there is no increased reactivity with monoclonal antibodies to activated T cells. There is controversy whether these abnormalities are more pronounced in patients with GVHD.

Bone marrow transplant recipients are predisposed to develop a number of infectious complications (54). During the immediate posttransplant period, patients have granulocytopenia and are predisposed to bacterial and fungal infections. Mucosal herpes simplex infections are common during this period but seldom disseminate; prophylactic acyclovir may decrease the incidence of this complication (55). Infectious viral gastroenteritis may also occur (56). Following recovery of normal peripheral blood cell counts, patients remain predisposed to several opportunistic viral and parasitic infections. Herpes zoster infections are common within the first year of transplantation (57). These are generally confined to a single dermatome but fatal dissemination may occur. Treatment with acyclovir or adenine arabinoside is beneficial in patients with disseminated disease and may prevent dissemination in immunocompromised patients with localized involvement (58).

Interstitial pneumonitis is one of the major complications of marrow transplantation which occurs in 25%-60% of patients, usually within the first 4 months (20-22). One-half or more of the cases are associated with CMV infection. *Pneumocystis carinii* or other viruses such as adenovirus, ECHO virus, respiratory syncytial virus, and herpes varicella-zoster are involved in some cases. Factors important in the development of interstitial pneumonitis include exogenous CMV infection (probably via blood product transfusions), activation of latent endogenous CMV infection, drug- or radiation-related lung injury, GVHD, and posttransplant immunodeficiency (50,59,60). Winston et al (61) have reported an increased incidence of CMV infections and interstitial pneumonitis in patients receiving granulocyte transfusions. GVHD may contribute to the incidence or severity of pneumonitis by compromising immunologic recovery or by activating latent CMV infection. Increased T-lymphocyte reactivity to CMV and impaired pulmonary macrophage function during the first 3-6 months after transplantation have been reported. Recent data indicate that direct infection of pulmonary alveolar macrophages with CMV results in impaired phagocytic activity (62). The role of drugs and irradiation used in the pre- and post-transplant treatment regimen on the pathogenesis of interstitial pneumonitis is controversial. Patients receiving fractionated TBI may have a lower incidence of idiopathic interstitial pneumonitis than those receiving single-dose TBI (22). Other factors such as the radiation dose rate and posttransplant immunosuppression with methotrexate may also be important (20).

Several approaches to the prevention or treatment of interstitial pneumonitis have been evaluated. Prophylactic treatment with trimethoprim/sulfamethoxazole is effective in preventing *Pneumocystis carinii* pneumonia (63). Antiviral agents including vidarabine, acyclovir, and interferon have not been beneficial in preventing or treating CMV infections in marrow transplant recipients (64). Three trials have indicated that passive immunization with antiserum to CMV can modify or prevent serious CMV infections and reduce the incidence of interstitial pneumonitis (65-67). Another potential approach is to exclusively use blood products from CMV-negative blood donors for transfusions.

The high-dose chemotherapy and radiation treatment programs for bone marrow transplantation are potentially carcinogenic and may predispose to the development of secondary malignancies. Only a small number of second malignancies have been reported in the long-term survivors of marrow transplantation, including two cases of immunoblastic sarcoma arising in donor cells (68,69). The incidence of treatment-induced malignancies may increase with further follow-up, particularly for radiation-induced tumors that may have a long latent period such as thyroid carcinoma.

In summary, serious acute or chronic complications may occur following allogeneic bone marrow transplantation which are fatal in 30%-50% of patients transplanted for malignancy.

Bone Marrow Transplantation for Acute Leukemia

Marrow transplantation has been most extensively utilized for the treatment of patients with AML and ALL. In the initial studies, marrow transplantation was reserved for patients with relapsed or refractory disease. A number of pretransplant treatment regimens involving high-dose chemotherapy alone or chemotherapy and irradiation have been evaluated as summarized in table 1 (70-81). Most patients are treated with regimens involving high doses of cyclophosphamide with or without other chemotherapy, ≥ 1000-rad TBI, followed by bone marrow transplantation. For patients receiving syngeneic marrow transplantation, approximately 25% achieve prolonged disease-free survival; the major cause of treatment failure is relapse of leukemia (77,78). For recipients of allogeneic marrow transplants, approximately 30% achieve prolonged continuous complete remissions[3] with 5-10-year survival in approximately 10%. The major causes of treatment failure are resistant leukemia, GVHD, and interstitial pneumonitis. Similar results have been achieved in patients with either AML or ALL. There is no evidence that any combination of drugs and irradiation is more effective than high-dose TBI alone. More intensive regimens such as SCARI (6-thioguanine, cyclophosphamide, cytarabine, daunorubicin, and irridiation) (74), designed to have greater antileukemic activity, have been associated with a high rate of complications due to toxicity; overall survival has not been improved. Recently, attempts have been focused on escalating the dose of TBI utilizing fractionated schedules with some improvement in results in preliminary reports (79).

These survival results, although disappointing, indicate that intensive chemoradiotherapy and bone marrow transplantation can be curative in patients with otherwise refractory leukemia. There is little controversy that these results are superior to those achieved with conventional chemotherapy in patients with advanced disease. Recent data suggest that results may be improved by employing bone marrow transplantation as the initial treatment of leukemic relapse rather than attempting reinduction of remission with chemotherapy (82).

It is important to understand the mechanisms of leukemic recurrence following transplantation. In the overwhelming majority of patients who relapse after bone marrow transplantation, the leukemic cells have been shown to be of host origin, indicating failure of the treatment regimen to completely eradicate all the leukemic cells. Improvement in the treatment outcome for these patients requires the development of more effective antileukemic therapy. There have been five cases reported, however, in which the leukemia appears to have arisen in donor cells (83-88), indicating that under rare circumstances the leukemia may be "reinduced" following apparently effective treatment. There are alternative explanations of this phenomenon which must be considered, including microenvironmental or regulatory abnormalities or somatic cell hybridization between a recipient leukemia cell and normal donor cells.

Recurrence of leukemia following allogeneic bone marrow transplantation may be reduced by an antileukemic effect associated with GVHD. For patients transplanted while in relapse the rate of leukemic recurrence in allo-

---

[3] Actuarial analysis of patients at risk.

TABLE 1.—Results of allogeneic bone marrow transplantation for relapsed or refractory acute leukemia*

Treatment regimen	Relapse (%)	Survival (%)
Cyclophosphamide	100	0
BACT†	100	0
TBI	70	10
Cyclophosphamide-TBI	70	15
SCARI	30	10

*Actuarial results at 5 yrs.

†B = carmustine, A = cytarabine, C = cyclophosphamide, and T = 6-thioguanine.

geneic transplant recipients without GVHD is 65%-70%, which is similar to that following syngeneic transplantation. Patients with moderate to severe acute GVHD have a lower rate of recurrent leukemia, approximately 35%, indicating that GVHD may be associated with immunologically mediated graft-versus-leukemia (GVL) effect. Unfortunately, due to the morbidity of GVHD, overall survival is not improved (89,90). Weiden et al (91) reported that the GVL effect is more closely associated with chronic GVHD than acute GVHD (91). In mice, this GVL effect can be separated from GVHD; in man, the distinction is less clear. One potential approach to improving the outcome of transplantation would be to develop a technique to accentuate this GVL effect without inducing severe GVHD.

A major advance in the application of bone marrow transplantation to patients with acute leukemia involves the treatment of patients while in remission (92). The rationale for this approach includes treatment of the patients with a relatively low burden of malignant cells prior to the development of resistant leukemia. In addition, patients in remission are generally in better medical condition than patients with resistant leukemia and can better tolerate the intensive chemoradiotherapy regimen.

Over 500 patients with AML have received bone marrow transplants while in first complete remission (81,92-105). Most centers have limited transplantation to patients < 45 years of age who have an HLA identical donor. Patients were generally conditioned with high-dose cyclophosphamide with or without cytarabine and TBI or with high-dose busulfan and cyclophosphamide. Most studies report actuarial relapse rates ranging from 10% to 35% and actuarial survival rates ranging from 40% to 65% at 3-5 years as shown in figure 3. Treatment failure was primarily due to GVHD, interstitial pneumonitis, and leukemic relapse. Thomas and coworkers (97) and Dinsmore et al (103) reported that patient age was an important prognostic factor for treatment outcome, but an effect of age could not be documented in the analysis of the International Bone Marrow Transplant Registry (105). Patients transplanted from identical twin donors may have a higher rate of relapse; three of five patients receiving syngeneic transplants at UCLA have relapsed and similar data have been reported at other centers. This is consistent with the concept that a GVL effect of allogeneic transplantation may be important in preventing leukemic recurrence.

These results of bone marrow transplantation must be compared to the recent advances achieved with combination chemotherapy. A number of investigators have reported improved remission duration and survival utilizing regimens involving intensive consolidation and/or maintenance schedules (96,106-109). There is a wide range of reported results among centers, with actuarial disease-free survival rates reported from 20% to 50% at 4-5 years (fig 3). A number of investigators have reported prognostic factors that predicted for prolonged duration of remission such as morphologic subtype, cytogenetic data, presence of a preleukemic syndrome, and rate of cytoreduction with induction chemotherapy (110-112). The value of these prognostic factors is controversial. Although analysis of these variables allows description of groups with a relatively high or low risk of relapse, it is still not possible to reliably identify patients with a > 50% chance of long-term survival.

Four centers have reported preliminary results of controlled trials of bone marrow transplantation versus combination chemotherapy in patients with AML in first remission (96,98,113,114). Each study has shown a substantially lower relapse rate in patients receiving transplantation. At present, two trials with a 5-year follow-up show a survival advantage to transplantation; two trials do not show a significant difference in survival as yet but are still too early for critical analysis. It is important to note that in the trials where marrow transplantation appears superior, survival in the chemotherapy control group was much lower than the best results reported in the literature.

It is fair to conclude that high-dose chemoradiotherapy and bone marrow transplantation are a more effective antileukemic treatment than intensive chemotherapy alone, resulting in a substantially lower rate of leukemic relapse. Because of transplant-related complications, the difference in overall survival is much less prominent. Although young patients appear to fare better with transplantation in several studies, it is not clear that this is the case for patients > 30 years of age. Further studies are required to identify prognostic factors that would allow selection of patients who would best be treated either with chemotherapy alone or with bone marrow transplantation.

One setting in which bone marrow transplantation may be particularly useful is the treatment of patients with preleukemia or therapy-related AML. In these settings conventional therapy is often unsuccessful due to the failure of normal hematopoietic cells to recover after induction chemotherapy. Intensive marrow ablative therapy with bone marrow transplantation has been successful in preliminary studies in producing complete remission and restoring normal hematopoiesis (115,116).

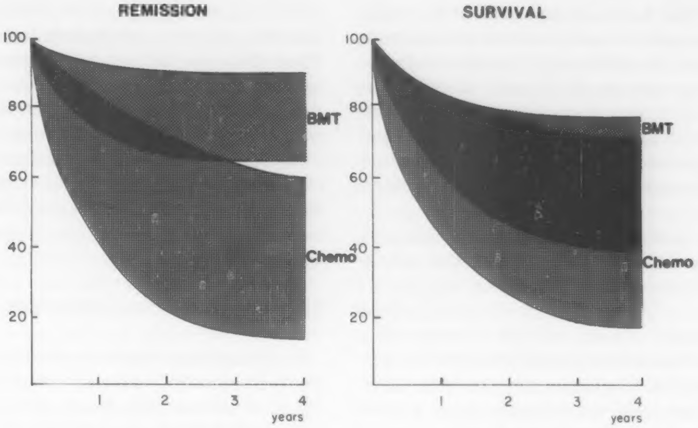


FIGURE 3.—Range of actuarial remission and survival curves reported for patients with AML in first complete remission receiving allogeneic bone marrow transplantation (BMT) vs combination chemotherapy alone (Chemo).

As an approach to patients who lack an HLA identical donor, a number of investigators have evaluated autologous bone marrow transplantation in patients with acute leukemia. Patients undergo procurement and cryopreservation of bone marrow cells while in remission. At a later time they may receive high-dose chemoradiotherapy and reinfusion of the cryopreserved hematopoietic cells. The major limitation of this approach is the high likelihood that the "remission" bone marrow may be contaminated with leukemic cells. These leukemic cells would also be cryopreserved and reinfused with the autologous marrow. In addition, since the patient receives autologous marrow cells, there is no possibility of a favorable GVL effect as described for allogeneic transplantation.

Over 50 patients with AML have received autologous marrow transplants while in relapse (117-120). As expected from the considerations described above, the major problem was rapid relapse of leukemia, the median duration of remission was 3-5 months, and < 10% of patients survived 1 year.

Recently, several centers have evaluated autologous marrow transplantation in patients with AML in first remission (121,122). Relapse of leukemia remains a major problem but approximately 20% have achieved a > 2-year disease-free survival. Although this result is not better than that achieved with standard chemotherapy alone, it does illustrate that this approach can be successful and that relapse of leukemia will not invariably result. A number of techniques have been explored to deplete occult leukemic cells from the remission bone marrow in vitro prior to cryopreservation. Immunologic approaches have been largely limited by cross-reactivity of most anti-AML antisera or monoclonal antibodies with normal hematopoietic progenitors. An additional limitation is the lack of a reliable assay to detect small numbers of occult leukemic cells. As yet, there is no convincing evidence that any in vitro technique to purge leukemic cells from remission marrow is effective in prolonging remissions or survival in patients receiving autologous marrow transplant for AML.

ALL in Remission

Over 400 patients with ALL have received bone marrow transplants while in first, second, or third remission. Since many newly diagnosed patients with ALL may achieve prolonged remission with conventional chemotherapy, transplantation has generally been reserved for patients in a second or greater remission. Patients have usually received conditioning regimens of high-dose cyclophosphamide and TBI (7.5-10 Gy in a single dose or up to 16 Gy in fractionated schedules).

There is general agreement that patients in their third or greater remission do poorly, with actuarial relapse rates of 45%-60% in most large series (123-127) and an actuarial 3-5-year survival rate of approximately 20%. These results are probably not significantly different than those observed with transplantation of patients in relapse. In addition, as a consequence of cumulative toxicity of previous antileukemic treatment these patients may be more susceptible to toxicity from the conditioning regimen. The results of transplantation for patients with ALL in second remission have been variable among major transplant centers, with actuarial relapse rates ranging from 25% to 60% and survival rates ranging from 30% to 50% at 3-5 years posttransplant. Attempts to improve this high relapse rate with more intensive conditioning using multiagent chemotherapy or high-dose fractionated TBI have not been successful (126). These results do compare favorably to those with conventional or experimental chemotherapy in patients who relapse while receiving maintenance therapy (23,125). Patients who relapse after maintenance has been discontinued and those with isolated extramedullary relapse have a much better prognosis with standard therapy; the role of marrow transplantation in these patients has not been established.

The role of bone marrow transplantation in patients with ALL in first remission is highly controversial. A number of centers have recently initiated trials of marrow transplantation in "high-risk" patients while in first remission. Preliminary results indicate relapse rates < 10% and disease-free survival rates of approximately 65% at 2 years (128,129). The major controversy involves the selection of "high-risk" patients who have a poor prognosis with conventional treatment. Patient age, initial wbc count, B- or T-cell surface phenotype, and cytogenetic data have been utilized as prognostic factors. Recently, however, a number of investigators have reported improved results with intensive induction and maintenance chemotherapy with 5-year disease-free survival ⩾ 50% in adults or children with poor prognostic features (130-132). Because of the considerable variability in the results of both chemotherapy and bone marrow transplantation in high-risk ALL, it is not possible to critically analyze their relative efficacy. Controlled clinical trials are required, with patients carefully stratified for known prognostic factors.

Autologous bone marrow transplantation has also been evaluated in patients with ALL. Unlike the situation in AML, a number of heteroantisera and monoclonal antibodies to ALL-associated antigens have been identified which are nonreactive with normal hematopoietic progenitors such as CFU-C, BFU-E, or CFU-GEMM (14). These include antibodies to the common ALL antigen (CALLA) and a number of T- or B-cell antigens (133,134). A number of patients have received autologous transplants utilizing bone marrow that was treated in vitro with one or more of these antibodies and complement (135-138). Although each of these antigens is expressed on subpopulations of normal lymphoid cells, engraftment has consistently occurred and immunologic recovery has not been adversely affected. Preliminary data suggest that in vitro treatment with multiple anti-ALL antibodies and complement may enhance the leukemic cell kill (139). Antibody-toxin conjugates may be more effective than relying on complement-mediated lysis (140). Limitations to this technique include the problem of antigenic heterogeneity among neoplastic cells and modulation of the target antigens off the leukemic cell surface.

Encouraging preliminary results have been presented for patients with ALL in second remission who received intensive chemotherapy, TBI, and autologous marrow transplantation utilizing marrow treated in vitro with anti-CALLA antibody and complement, with a number of patients achieving remissions lasting > 2 years (135). These data are difficult to interpret since many of the successful cases involved patients with a relatively good prognosis with conventional treatment, such as patients who relapse after a long first remission and in whom maintenance therapy has been discontinued. Controlled clinical trials are required to compare this treatment with the best available standard chemotherapy for patients with ALL who have relapsed while receiving maintenance therapy.

Chronic Myelogenous Leukemia

Encouraging preliminary results have recently been reported in the application of bone marrow transplantation for the treatment of patients with CML. CML is a hematologic malignancy characterized by excessive proliferation of myeloid cells and their progenitors. In > 90% of cases, the Philadelphia chromosome (t9,22) is a marker of the malignant clone. The disease is characterized by two phases, an initial *chronic phase* in which cell maturation is normal, followed by transformation to *acute phase* (blastic crisis) characterized by maturation arrest at the level of the myeloblast or lymphoblast (141). Some patients develop a transient accelerated phase prior to transformation to overt blast crisis. Median survival of patients with CML is approximately 3-4 years (142). CML is a universally fatal disease with conventional therapy. Treatment with alkylating agents or hydroxyurea can control the growth of the malignant cells during the chronic phase but is incapable of eradicating the leukemia. Once the disease transforms to acute phase, conventional treatment is of limited benefit and median survival is < 6 months (143).

A major obstacle to the successful treatment of CML is that there appears to be no differential sensitivity of the leukemic cells over normal hematopoietic cells to cytotoxic chemotherapy (144). Thus, intensive treatment attempting to eradicate CML is likely to produce unacceptable bone marrow toxicity. To circumvent this problem, several investigators have evaluated the use of intensive marrow ablative doses of chemotherapeutic drugs and radiation followed by transplantation of normal hematopoietic cells (145). Three sources of hematopoietic cells have been utilized: autologous cryopreserved peripheral blood or bone marrow cells, syngeneic bone marrow cells from a genetically identical twin, or allogeneic bone marrow from an HLA identical sibling donor. Most patients have received high-dose cyclophosphamide, with or without cytarabine or other chemotherapy, followed by 5-15 Gy of TBI either as a single dose or fractionated over 3-7 days. A small number of patients have been treated with the combination of high-dose busulfan and cyclophosphamide. Recipients of allogeneic transplants received either methotrexate or cyclosporine following the transplant to prevent or modify GVHD.

For autologous transplants in CML, a large number of peripheral blood and/or bone marrow hematopoietic cells are collected and cryopreserved during the chronic phase of the disease (146). Patients who enter blast crisis may then receive marrow ablative chemotherapy and/or radiation followed by infusion of the cryopreserved autologous stem cells. The objective of this approach is to re-establish the chronic phase of the disease. The procedure has palliative intent. Since the transplanted bone marrow contains Ph^1-positive leukemic cells, *cure* of the disease is not possible. In studies reported to date, this approach has provided only limited palliative benefit. Most patients will achieve a second chronic phase but early relapse of blast crisis has been the major problem. Median duration of remission is < 4 months. Median survival is approximately 6 months and only 10% survive 2 years (147,148). These results are not substantially different than those reported for the treatment of acute phase with conventional chemotherapy.

With syngeneic or allogeneic bone marrow transplantation, the objective is to cure the patient's CML by eradication of the leukemic clone of cells with very intensive marrow ablative chemoradiotherapy and restoration of hematopoiesis by transplantation of normal donor stem cells. Following the original report of Fefer and co-workers (149), a number of centers have evaluated syngeneic bone marrow transplantation in patients with CML (150–152). If patients are transplanted while in blast crisis, leukemic relapse is a major problem occurring in approximately 70% of patients. Approximately 20% of patients have survived free of disease > 5 years, demonstrating that the intensive marrow ablative therapy can eradicate this disease in some patients. These results are similar to those achieved for patients with acute leukemia transplanted while in relapse. Better results have been reported for patients receiving syngeneic bone marrow transplantation while in chronic phase; the actuarial remission rate and survival are each approximately 60% at > 5 years.

Over 300 patients with CML have received allogeneic marrow transplantation from an HLA identical donor (153–162) (fig 4). Patients transplanted in acute phase have fared poorly with results similar to those in patients receiving bone marrow transplantation for acute leukemia in relapse. The actuarial relapse rate is approximately 60%. Median survival is only 2 months, but 10% survive > 5 years in continuous remission. Patients transplanted while in an accelerated phase have had somewhat better results; the actuarial relapse rate is approximately 30% and the 3-year survival is 35%. Engraftment is delayed and platelet transfusion is often difficult in patients with massive splenomegaly, leading some centers to routinely perform splenectomies in transplant candidates.

The best results have been reported with allogeneic bone marrow transplantation for patients in chronic phase. In studies involving > 80 patients, actuarial disease-free survival is 60%–70% at 3 years, and < 5% of patients have relapsed. Most relapses have been identified by routine cytogenetic studies in asymptomatic patients and it is likely that the relapse rate will increase with further follow-up of these patients. A small number of patients who entered blast crisis but achieved a second chronic phase with intensive chemotherapy were transplanted at that time. These patients have surprisingly good results in preliminary studies; approximately one-half of this group have achieved prolonged disease-free survival.

These results for bone marrow transplantation in CML must still be considered preliminary. Most patients are within 2 years of transplantation, and further studies with long-term follow-up are required. A number of tentative conclusions can be drawn from these data. Patients with CML in blast crisis or a well-defined accelerated phase have a poor prognosis with conventional treatment; median survival is < 3 months and few patients survive 1 year. Allogeneic and syngeneic bone marrow transplantation are generally unsuccessful in these patients; median survival is not improved but a small proportion may achieve long-term disease-free survival. Results have been better in patients transplanted in chronic phase, before transformation of their disease. The optimal timing of transplantation in patients in chronic phase is controversial. The risks and potential benefits of early bone marrow transplantation must be balanced against the risk of delaying the treatment, ie, transformation of the leukemia. For patients who are clinically stable, there is a relatively constant risk of transformation to acute phase; approximately 25% of patients surviving at any point will transform to acute phase during the ensuing year and die of their disease (142). Recently, a number of investigators have reported prognostic factors that may identify good-, average-, and poor-risk groups when treated with conventional treatment (163,164). Figure 5 compares survival of patients receiving bone marrow transplantation in chronic phase versus these three prognostic groups as defined by Tura et al (163). Early bone marrow transplantation appears justified in patients in average and poor prognostic groups, whereas a conservative approach may be more appropriate in good-prognosis patients (those with an initial low wbc count, small liver and spleen, and easily controlled with chemotherapy). It may be advisable to delay transplantation in these patients until the development of adverse clinical findings.

Bone Marrow Transplantation From Other Than HLA Identical Siblings

With the apparent success of allogeneic bone marrow transplantation from an HLA identical donor for selected patients with acute and chronic leukemia, a number of investigators have evaluated the use of a donor and recipient who are not completely identical for the major histocompatibility loci. Clift et al (165) reported that donor-recipient pairs who are mismatched for only one A, B, or D HLA locus or HLA phenotypically identical parent-child pairs had results indistinguishable from transplantation between HLA-MLC genotypically identical siblings; patients mismatched for two or more loci had poorer results. Powles et al (166) reported results of patients receiving transplantation from haploidentical donors, most of whom were mismatched for two or three HLA A, B, or D loci. Most patients received the combination of methotrexate and cyclosporine posttransplant. Patients < 20 years of age had survival similar to those with HLA identical donors, although 80% had moderate to severe acute GVHD. There were no survivors in recipients > 20 years of age (166).

A number of investigators are evaluating the use of haploidentical donors, with the donor bone marrow being treated in vitro to deplete mature T lymphocytes with the objective of preventing GVHD. These studies were begun too recently for critical analysis, and this approach must be considered highly experimental.

Another means to expand the donor pool would be to identify HLA identical, unrelated donors from the general population. A small number of successful unrelated transplants have been performed (167). The major limitation of this approach is the logistic considerations in typing and maintaining a large library of potential donors. A listing of > 50,000 people is required to provide an HLA-mixed lymphocyte culture identical donor for 80% of recipients (168).

Bone Marrow Transplantation for Lymphoma and Solid Tumors

Based on the success of high-dose combined modality therapy and bone marrow transplantation for patients with leukemia, a number of investigators have evaluated a similar approach in the treatment of lymphomas and a number of solid tumors. Several comprehensive reviews in this area are available (4,5,169,170).

Syngeneic, allogeneic, and autologous marrow transplants have been studied in patients with non-Hodgkin's lymphoma. Most patients have been transplanted for high-grade lymphomas (Burkitt's lymphoma, lymphoblastic lymphoma, or diffuse histiocytic lymphomas) that had failed to respond or relapsed after treatment with standard combination chemotherapy. Eleven patients with advanced lymphomas who had failed conventional treatment have received high-dose chemoradiotherapy with cyclophosphamide and TBI with or without other chemotherapy, followed by syngeneic marrow transplantation (171–174). Five patients have achieved prolonged remissions, four have relapsed, and two died of treatment-related complications. Similar results have been reported in a small number of young patients receiving allogeneic marrow transplants (175). These data indicate that these very intensive treatment regimens are capable of eradicating the disease, even in patients refractory to standard treatment.

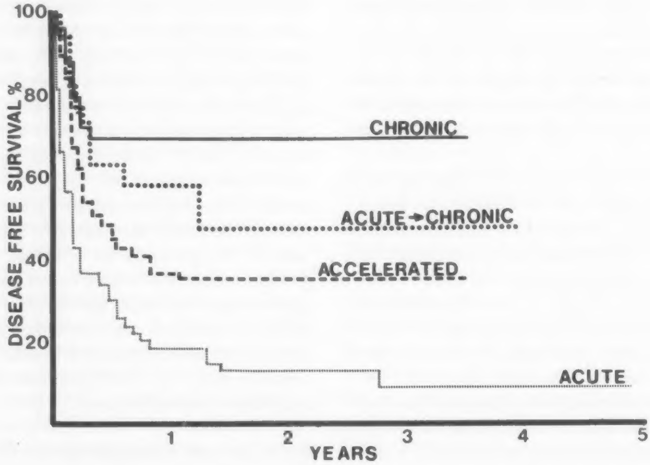


FIGURE 4.—Representative disease-free survival curves for patients receiving bone marrow transplantation for CML in chronic phase, accelerated phase, or acute phase, or in patients who entered acute phase but achieved a second chronic phase with chemotherapy (acute → chronic) (*from* ref 145).

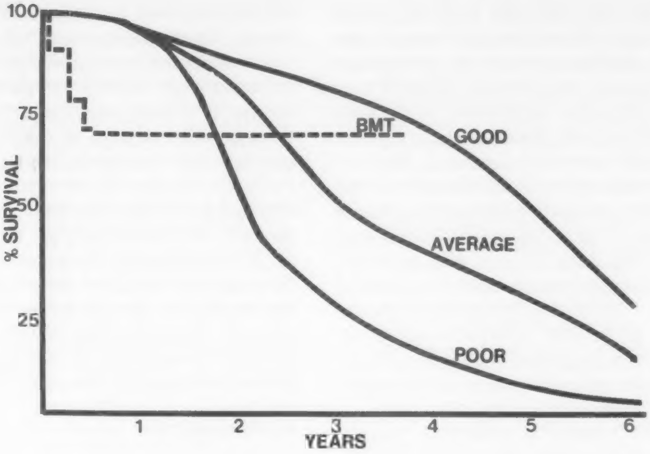


FIGURE 5.—Comparison of survival following bone marrow transplantation vs conventional treatment of patients in good, average, or poor prognostic groups (*from* ref 145).

Autologous bone marrow transplantation has been more extensively studied in patients with resistant or recurrent lymphoma. Patients have received either intensive chemotherapy alone such as BACT or TACC (carmustine or lomustine, cytarabine, cyclophosphamide, and 6-thioguanine) (176,177) or cyclophosphamide/TBI-based regimens (178,179). Results have been highly variable. Most patients achieve complete remissions with this treatment and approximately one-third of the patients survive in remission for > 1 year. In patients with Burkitt's lymphoma or diffuse large cell lymphomas, toxicity has been a major problem, particularly in heavily pretreated and debilitated patients. Fatal hepatic, cardiac, and pulmonary toxicity have been reported. Results have been relatively poor in patients with lymphoblastic lymphoma; relapse has been the major problem. Since this disease is similar to T-cell acute lymphoblastic leukemia and has a marked tendency to involve the bone marrow, these failures are likely to be related to occult bone marrow involvement with lymphoma at the time of cryopreservation. Recently, clinical trials have been initiated to evaluate techniques to purge the marrow of contaminating lymphoma cells utilizing antibodies to T- or B-cell antigens or in vitro treatment with chemotherapy (137).

Marrow transplantation may be particularly useful in the treatment of several pediatric tumors such as neuroblastoma, Ewing's sarcoma, and other tumors which are highly sensitive to a number of chemotherapeutic agents or radiation yet have a poor prognosis in patients with advanced disease.

Patients with far-advanced or recurrent neuroblastoma have a dismal prognosis with standard treatment (180). Several studies are currently in progress utilizing high-dose combined modality therapy and allogeneic marrow transplantation or autologous marrow transplantation in these patients. Neuroblastoma frequently involves the bone marrow. A number of monoclonal antibodies reactive with neuroblastoma-related antigens have been described (180) and may be useful in detecting microscopic bone marrow involvement and for purging of metastatic neuroblastoma cells from the marrow in vitro prior to cryopreservation. High-dose melphalan with autologous marrow transplantation has produced complete remissions in approximately one-half of patients with advanced and relapsed disease and several patients have remained free of disease > 1 year (181,182,186). Other centers have reported encouraging preliminary results with more intensive pretransplant conditioning regimens involving combination chemotherapy and TBI with either allogeneic or autologous marrow transplantation (183).

Ewing's sarcoma is another potential candidate tumor for autologous marrow transplantation. It is responsive to chemotherapy and rarely involves the bone marrow. High-dose melphalan (181,184,185) or cyclophosphamide/TBI (172) with autologous bone marrow transplantation can produce complete remissions in patients with relapsed Ewing's sarcoma, but these responses are usually brief. In preliminary studies, intensive chemoradiotherapy with autologous bone marrow transplantation may be more successfully utilized as consolidation treatment as part of first-line therapy (185).

A similar approach with autologous marrow transplantation has been utilized for the treatment of Hodgkin's disease, testicular and other germ cell carcinomas, and small cell carcinoma of the lung (172,177,187,188). A variety of intensive treatment regimens have been utilized. Although most patients have achieved a complete or partial response, durable remissions have only rarely been achieved in patients with relapsed and resistant disease. Severe hepatic, cardiopulmonary, or gastrointestinal toxicity has frequently complicated the use of very intensive treatment regimens, particularly in patients who are debilitated due to their disease or to the cumulative toxicity of extensive previous treatment. More recent studies are focusing on use of this modality in the adjuvant setting or as consolidation therapy in patients at high risk to relapse after initial cytoreduction with standard treatment (189). As is the case with acute leukemia and a number of animal models (190), treatment of patients in remission with relatively low tumor burden may be more likely to produce durable complete responses. In addition, the treatment would be administered at a point when the patient is in good clinical condition and able to tolerate intensive therapy.

Improvement in the results of autologous marrow transplantation for solid tumors requires the development of effective treatment regimens which are not excessively toxic to normal tissues. A number of chemotherapeutic agents can be escalated to two to five times their standard dosage in the setting of autologous marrow transplantation including cyclophosphamide, melphalan, busulfan, carmustine, lomustine, mitomycin, etoposide, and amsacrine (186,191,193-197,198). Further phase I-II clinical studies are required to determine the efficacy and nonhematopoietic toxicity for other chemotherapeutic agents in high doses. Candidate drugs shuld have some efficacy in standard dosage, have toxicity limited primarily to the bone marrow, and lack substantial nonmarrow toxicity. Hopefully, these single-agent studies will form the basis for potentially more effective combination regimens.

A number of malignancies that are poorly responsive to conventional treatment have been treated with high-dose chemotherapy and autologous marrow transplantation, including melanoma (186,188), breast (172,192), colon and other gastrointestinal carcinomas (192), and glioblastoma multiforme (194). These patients have generally received escalated doses of single agents such as melphalan, carmustine, mitomycin, or amsacrine. This high-dose treatment has increased the proportion of patients achieving complete and partial responses but these responses are usually brief and it is unclear if overall survival is improved. Controlled clinical trials are required to assess the efficacy of this treatment approach versus conventional management of those patients.

CONCLUSION

In summary, the doses of myelotoxic antineoplastic drugs and radiation can be escalated to more effective levels if followed by bone marrow transplantation to prevent severe marrow suppression. A number of malignancies have been successfully treated with this approach, particularly acute leukemias, CML, and lymphomas. Although the role of bone marrow transplantation is well-established for selected groups of patients, the efficacy of transplantation must be compared to recent advances in treatment of many neoplasms with conventional chemotherapy and radiation in controlled clinical trials. Improvement in the results with transplantation requires evolution of more effective but tolerable pretransplant chemoradiotherapy treatment programs and measures to prevent transplant-related complications, primarily GVHD, immunodeficiency, and interstitial pneumonitis in allogeneic transplant recipients. The success of autologous marrow transplantation will depend on development of effective means to detect and selectively eradicate microscopic marrow metastases without toxicity to the normal hematopoietic cells.

REFERENCES

1. FREI E, and CANELLOS GP. Dose: a critical factor in cancer chemotherapy. Am J Med 69:585-594, 1980.
2. THOMAS ED. The role of bone marrow transplantation with eradication of malignant disease. Cancer 10:1963-1969, 1982.
3. GALE RP. Progress in bone marrow transplantation in man. Surv Immunol Res 1:40-66, 1982.
4. DIESSEROTH A, and ABRAMS RA. The role of autologous stem cell reconstitution in intensive therapy for resistant neoplasms. Cancer Treat Rep 63:461-471, 1979.
5. GALE RP. Autologous bone marrow transplantation in patients with cancer. JAMA 243:540-542, 1980.
6. THOMAS ED, STORB R, CLIFT RA, ET AL. Bone marrow transplantation. N Engl J Med 292:832-843, 895-902, 1975.
7. APPELBAUM FR, HERZIG GP, GRAW RG, ET AL. Study of cell dose and storage time on engraftment of cryopreserved autologous bone marrow in canine model. Transplantation 26:245-248, 1978.
8. THOMAS ED, RAMBERG ER, SALE GE, ET AL. Direct evidence for a bone marrow origin of the alveolar macrophage in man. Science 192:1016, 1976.
9. GALE RP, SPARKES RS, and GOLDE DW. Bone marrow origin of hepatic macrophage (Kupffer cells) in man. Science 201:937-938, 1978.
10. COCCIA PF, KRIVIT W, CERVENKA J, ET AL. Successful bone marrow transplantation for infantile malignant osteopetrosis N Engl J Med 302:701-708, 1980.
11. SPARKES RS, SPARKES MC, GALE RP, ET AL. Immunoglobulin synthesis following allogeneic bone marrow transplantation in man: conversion to donor allotype. Transplantation 27:212-213, 1979.
12. SINGER JW, KEATING A, and FIALKOW PJ. Evidence suggesting a common progenitor for hemotopoietic and marrow stromal cells. Exp Hematol 11(supp 14):4, 1983.
13. WELLS JR, SULLIVAN A, and CLINE MJ. A technique for separation and cryopreservation of myeloid stem cells from human marrow. Cryobiology 16:201, 1979.
14. FOON K, SCHROFF R, and GALE RP. Surface markers on leukemia and lymphoma cells: recent advances. Blood 60:1-19, 1982.
15. BAST RC, RITZ J, LIPTON JM, ET AL. Elimination of leukemic cells from human bone marrow using monoclonal antibody and complement. Cancer Res 43:1389-1394, 1983.
16. KROLICK A, UHR J, and VITETTA E. Selective killing of leukemia cells by antibody-toxin conjugate: implications for autologous bone marrow transplantation. Nature 295:604, 1982.
17. SHARKIS SJ, SANTOS GW, and COLVIN M. Elimination of acute myelogenous leukemia cells from marrow and tumor suspension in

the rat with 4 hydroperoxycyclophosphamide. Blood 55:521-523, 1980.

18. KEMSHEAD JT, GIBSON FG, UGELSTAD J, ET AL. A flow system for the in vitro separation of tumor cells from bone marrow using monoclonal antibodies and magnetic microspheres. Proc Am Assoc Cancer Res 24:857, 1983.
19. SHULMAN HM, MCDONALD GB, MATTHEWS D, ET AL. An analysis of hepatic venoocclusive disease and centrilobular hepatic degeneration following bone marrow transplantation. Gastroenterology 79:1178-1191, 1980.
20. BORTIN MM, KAY HEM, GALE RP, ET AL. Factors associated with interstitial pneumonitis after bone marrow transplantation for acute leukemia. Lancet 1:437-439, 1982.
21. WINSTON DJ, BRYSON YJ, HO WG, ET AL. Interstitial pneumonia and cytomegalovirus infection after bone marrow transplantation. *In* The Biology of Bone Marrow Transplantation (Gale RP, and Fox CF, eds). New York, Academic Press, 1980, p 83.
22. MEYERS JD, FLOURNOY N, and THOMAS ED. Non bacterial pneumonia after allogeneic marrow transplantation: a review of ten years experience. Rev Infect Dis 4:1119-1132, 1982.
23. JOHNSON FL, THOMAS ED, CLARK BS, ET AL. A comparison of marrow transplantation with chemotherapy for children with acute lymphoblastic leukemia in second or subsequent remission. N Engl J Med 305:846-851, 1981.
24. SKLAR CA, KIM TH, and RAMSEY NKC. Thyroid dysfunction among long term survivors of bone marrow transplantation. Am J Med 73:688-694, 1982.
25. SANDERS J. Effects of cyclophosphamide and total body irradiation on ovarian and testicular function. Exp Hematol 10(supp 11):49, 1982.
26. VAN BEKKUM DW. Immunologic basis of graft-versus-host disease. *In* The Biology of Bone Marrow Transplantation (Gale RP, and Fox CF, eds). New York, Academic Press, 1980, pp 175-194.
27. GLUCKSBERG H, STORB R, FEFER A, ET AL. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA matched sibling donors. Transplantation 18:295-304, 1974.
28. HERSHKO C, and GALE RP. GVHD scoring system for predicting survival of specific mortality in bone marrow transplant recipients. *In* The Biology of Bone Marrow Transplantation (Gale RP, and Fox CF, eds). New York, Academic Press, 1980, pp 59-68.
29. RAPPEPORT J, MIHM M, REINHERZ EL, ET AL. Acute graft-versus-host disease in recipients of bone marrow transplants from identical twin donors. Lancet 2:717-720, 1979.
30. POWLES RL, CLINK HM, SPENCE D, ET AL. Cyclosporin A to prevent graft-versus-host disease in man after allogeneic bone marrow transplantation. Lancet 1:327-329, 1980.

30a. RAMSEY NKC, KERSEY JH, ROBINSON LL, ET AL. A randomized study of the prevention of acute graft-versus-host disease. N Engl J Med 306:392-397, 1982.

31. DONEY KC, WEIDEN PL, STORB R, ET AL. Treatment of graft-versus-host disease in human allogeneic marrow graft recipients. A randomized trial comparing antithymocyte globulin and corticosteroids. Am J Hematol 11:1-8, 1981.
32. POWLES RL, BARRETT AJ, CLINK HM, ET AL. Cyclosporin A for the treatment of graft-versus-host disease in man. Lancet 2:1327-1331, 1978.
33. DICKE KA, VAN HOOFT JIM, and VAN BEKKUM DW. The selective elimination of immunologically competent cells from bone marrow and lymphatic cell mixtures. Transplantation 6:562-570, 1968.
34. REISNER Y, O'REILLY RJ, KAPOOR N, ET AL. Allogeneic bone marrow transplantation using stem cells fractioned by lectins: VI, in vitro analysis of human and monkey bone marrow cells fractionated by sheep blood cells and soybean agglutinin. Lancet 2:1320-1324, 1980.
35. PRENTICE HG, GANESHAGURU K, BRADSTOCK KF, ET AL. Remission induction with adenosine-deaminase inhibitor 2′-deoxycoformycin in thy-lymphoblastic leukemia. Lancet 1:170-172, 1980.
36. BILLING R, WELLS JR, GALE RP, ET AL. In vitro treatment of bone marrow with heteroantisera prior to transplantation. *In* The Biology of Bone Marrow Transplantation (Gale RP, and Fox CF, eds). New York, Academic Press, 1980, pp 167-174.
37. HAAS RJ, JANKA G, NETZEL B, ET AL. Antibody incubation of human bone marrow graft for prevention of graft-versus-host disease. Blut 40:337-397, 1980.
38. PRENTICE HG, BLACKLOCK HA, JANOSSY G, ET AL. Use of anti T cell monoclonal antibody to prevent acute graft-versus-host disease in allogeneic bone marrow transplantation for acute leukemia. Lancet 1:700-703, 1982.
39. SHULMAN HM, SULLIVAN KM, WEIDEN PL, ET AL. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. Am J Med 69:204-217, 1980.
40. STORB R, PRENTICE RC, SULLIVAN KM, ET AL. Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA identical siblings. Ann Intern Med 98:461-465, 1983.
41. FURST DE, CLEMENTS PJ, GRAZE PR, ET AL. Syndrome resembling progressive systemic sclerosis (PSS) following bone marrow transplantation—a model for PSS? Arthritis Rheum 22:904-910, 1979.
42. GRAZE PR, and GALE RP. Chronic graft-versus-host disease: a syndrome of disordered immunity. Am J Med 66:611-620, 1979.
43. TSOI MS, STORB R, DOBBS S, ET AL. Nonspecific suppressor cells in patients with chronic graft-versus-host disease after marrow grafting. J Immunol 123:1970-1976, 1979.
44. SULLIVAN KM, SHULMAN HM, STORB R, ET AL. Chronic graft-versus-host disease in fifty-two patients: adverse natural course and successful treatment with combination immunosuppression. Blood 57:267-276, 1981.
45. GALE RP, OPELZ G, MICKEY MR, ET AL. Immunodeficiency following allogenic bone marrow transplantation. Transplant Proc 10:223-227, 1978.
46. NOEL DR, WITHERSPOON RP, STORB R, ET AL. Does graft-versus-host disease influence the tempo of immunologic recovery after allogeneic human marrow transplantation? An observation of 56 long-term survivors. Blood 51:1087-1105, 1978.
47. SANTOS GW, ELFENBEIN GJ, SHARKIS S, ET AL. Reconstitution of lymphohematopoietic function following allogeneic, syngeneic, and autologous marrow transplantation. *In* Biological Basis of Immunodeficiency (Gelfan, and Dosch, eds). New York, Raven Press, 1980, pp 93-306.
48. CLARK RA, JOHNSON FL, KLEBANOFF SJ, ET AL. Defective neutrophil chemotaxis in bone marrow transplant patients. J Clin Invest 58:22-31, 1976.
49. WITHERSPOON RP, KOPECKY K, STORB R, ET AL. Immunological recovery in 48 patients following syngeneic marrow transplantation for hematological malignancy. Transplantation 33:143, 1982.
50. SCHROFF RW, GALE RP, and FAHEY JL. Immune restoration following bone marrow transplantation: lymphoid cell immaturity and immunoregulatory imbalance. J Immunol 129:1926-1930, 1982.
51. ATKINSON K, GOEHLE S, HANSEN J, ET AL. Human T-cell subpopulations identified by monoclonal antibodies after bone marrow transplantations. Exp Hematol 9(suppl):188, 1981.
52. FREIDRICH W, O'REILLY RJ, KOZINER B, ET AL. Human T cell subset development following marrow transplantation. Exp Hematol 9(suppl):188, 1981.
53. GOTTLIEB MS, SCHROFF R, SCHANKER HM, ET AL. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual man. Evidence of a new severe acquired cellular immunodeficiency. N Engl J Med 305:1425-1431, 1981.
54. WINSTON DJ, GALE RP, MEYER DV, ET AL. Infectious complications of human bone marrow transplantation. Medicine 58:1-31, 1979.
55. SARAL R, BURNS WH, LASKIN OL, ET AL. Acyclovir prophylaxis of herpes simplex virus infections: a randomized, double blind, controlled trial in bone marrow transplant recipients. N Engl J Med 305:63-67, 1981.
56. YOLKEN RH, BISHOP CA, TOWNSEND TR, ET AL. Infectious gastroenteritis in bone marrow transplant recipients. N Engl J Med

306:1009-1012, 1982.

57. ATKINSON K, MEYERS JD, STORB R, ET AL. Varicella-zoster virus infection after marrow transplantation for aplastic anemia or leukemia. Transplantation 29:47-50, 1980.
58. BALFOUR HH, BEAN B, LASKIN OL, ET AL. Acyclovir halts progression of herpes zoster in immunocompromised patients. N Engl J Med 308:1448-1453, 1983.
59. BRYSON Y, GARD S, ANK B, ET AL. Studies of cellular immunity to cytomegalovirus and lymphokine production by lymphocytes of bone marrow transplant recipients. *In* The Biology of Bone Marrow Transplantation (Gale RP, and Fox CF, eds). New York, Academic Press, 1980, pp 321-328.
60. WITHERSPOON RP, STORB R, MICKELSON EM, ET AL. Association of interstitial pneumonia and diminished in vitro lymphocyte blastogenesis in human marrow graft recipients. Transplantation 28:412-414, 1979.
61. WINSTON DJ, HO WG, HOWELL CL, ET AL. Cytomegalovirus infections associated with leukocyte transfusions. Ann Intern Med 93:671-675, 1980.
62. WINSTON DJ, TERRITO MC, HO WG, ET AL. Alveolar macrophage dysfunction in human bone marrow transplant recipients. Am J Med 73:859-866, 1982.
63. WINSTON DJ, LAU WK, GALE RP, ET AL. Trimethoprim-sulfamethoxazole for the treatment of *Pneumocystis carinii* pneumonia. Ann Intern Med 92:762-769, 1980.
64. WINSTON DW. Prevention and treatment of cytomegalovirus infection in bone marrow transplant recipients. *In* Recent Advances in Bone Marrow Transplantation (Gale RP, ed). New York, Alan Liss, 1983, pp 425-444.
65. WINSTON DW, POLLARD RB, HO WG, ET AL. Cytomegalovirus immune plasma in bone marrow transplant recipients. Ann Intern Med 97:11-18, 1982.
66. MEYERS JD, LESZCYNSKI J, ZAIA JA, ET AL. Prevention of cytomegalovirus infection by cytomegalovirus immune globulin after marrow transplantation. Ann Intern Med 98:442-446, 1983.
67. O'REILLY RJ, REICH L, GOLD J, ET AL. A randomized trial of intravenous hyperimmune globulin for the prevention of cytomegalovirus (CMV) infections following marrow transplantation: preliminary results. Transplant Proc 15:1405-1411, 1983.
68. GOSSETT TC, GALE RP, FLEICHMAN H, ET AL. Immunoblastic sarcoma in donor cells after bone marrow transplantation. N Engl J Med 300:904-907, 1979.
69. SCHUBACH WH, HACKMAN R, NEIMAN PE, ET AL. A monoclonal immunoblastic sarcoma in donor cells bearing Epstein Barr virus genomes following allogeneic grafting for acute lymphoblastic leukemia. Blood 60:180-187, 1982.
70. THOMAS ED, BUCKNER CD, BANAJI M, ET AL. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation and allogeneic marrow transplantation. Blood 49:511-513, 1977.
71. SANTOS GW, SENSENBRENNER LL, ANDERSON PN, ET AL. HLA-identical marrow transplants in aplastic anemia, acute leukemia, and lymphosarcoma employing cyclophosphamide. Transplant Proc 8:607-610, 1976.
72. GRAW RG, JR, YANKEE RA, ROGENTINE GN, ET AL. Bone marrow transplantation from HLA-matched donors to patients with acute leukemia. Transplantation 14:79-90. 1972.
73. UCLA BONE MARROW TRANSPLANTATION TEAM. Bone marrow transplantation in acute leukemia. Lancet 2:1197-1200, 1977.
74. UCLA BONE MARROW TRANSPLANTATION TEAM. Bone marrow transplantation with intensive combination chemotherapy-radiation therapy (SCARI) in acute leukemia. Ann Intern Med 86:155-159, 1977.
75. BORTIN MM, and RIMM AA. Bone marrow transplantation for acute myeloblastic leukemia. JAMA 1245-1252, 1978.
76. FORMAN SJ, SPRUCE WE, FARBSTEIN MJ, ET AL. Bone marrow ablation followed by allogeneic marrow grafting during the first complete remission of acute nonlymphocytic leukemia. Blood 61:439-442, 1983.
77. FEFER A, BUCKNER CD, THOMAS ED, ET AL. Cure of hematologic neoplasia with transplantation of marrow from identical twins. N Engl J Med 297:146-148, 1977.
78. FEFER A, CHEEVER MA, THOMAS ED, ET AL. Bone marrow transplantation for refractory acute leukemia in 34 patients with identical twins. Blood 57:421-438, 1981.
79. BADGER C, BUCKNER CD, THOMAS ED, ET AL. Allogeneic marrow transplantation for acute leukemia in relapse. Leukemia Res 6:383-388, 1982.
80. BORTIN MM, GALE RP, and RIMM AA. Factors associated with early mortality following allogeneic bone marrow transplantation for acute myelogenous leukemia: a report from the International Bone Marrow Transplant Registry. Transplant Proc 15:1392, 1983.
81. SANTOS GW, TUTSCHKA, BESCHORNER WE, ET AL. Marrow transplantation in acute non-lymphocytic leukemia following busulfan and cyclophosphamide. Blood 58(supp 1):176a, 1981.
82. APPELBAUM FR, CLIFT RA, BUCKNER CD, ET AL. Allogeneic marrow transplantation for acute non-lymphoblastic leukemia after first relapse. Blood 61:949-953, 1983.
83. FIALKOW PJ, THOMAS ED, BRYANT JI, ET AL. Leukaemic transformation of engrafted human marrow cells in vivo. Lancet 1:251-255, 1971.
84. THOMAS ED, BRYANT JI, BUCKNER CD, ET AL. Leukaemic transformation of engrafted human cells in vivo. Lancet 1:1310-1313, 1972.
85. ELFENBEIN GJ, DIGAMBER SB, BIAS WB, ET AL. Cytogenetic evidence for recurrence of acute myelogenous leukemia after allogeneic bone marrow transplantation in donor hematopoietic cells. Blood 52:627-636, 1978.
86. GOH K, and KLEMPERER MR. In vivo leukemic transformation: cytogenetic evidence of in vivo leukemic transformation of engrafted marrow cells. Am J Hematol 2:283-290, 1977.
87. NEWBURGER PE, LATT SA, PESANDO JM, ET AL. Leukemia relapse in donor cells after allogeneic bone marrow transplantation. N Engl J Med 304:712-714, 1981.
88. BOYD CN, RAMBERG RC, and THOMAS ED. The incidence of recurrence of leukemia in donor cells after allogeneic bone marrow transplantation. Leukemia Res 6:833-837, 1982.
89. WEIDEN PL, FLOURNOY N, THOMAS ED, ET AL. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic marrow grafts. N Engl J Med 300:1068-1073, 1979.
90. MCINTYRE R, and GALE RP. Graft-versus-leukemia in man. *In* Graft-Versus-Leukemia (Kunewick JP, and Meredith RF, eds). Florida, CRC Press, 1982, pp 1-10.
91. WEIDEN PL, SULLIVAN KM, FLOURNOY N, ET AL. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med 304:1529-1532, 1981.
92. THOMAS ED, BUCKNER CD, CLIFT RA, ET AL. Marrow transplantation for acute nonlymphoblastic leukemia in first remission. N Engl J Med 301:597-599.
93. ZWAAN FE. Bone marrow transplantation for acute leukaemia in remission—European results. Blut 41:208-213, 1980.
94. MORGENSTERN GR. Allogeneic bone marrow transplantation for acute leukaemia. Experience in 55 patients. Blut 41:213-215, 1980.
95. BLUME KG, BEUTLER E, BROSS KJ, ET AL. Bone marrow ablation and allogeneic marrow transplantation in acute leukemia. New Engl J Med 302:1041-1046, 1980.
96. CHAMPLIN R, ZIGHELBOIM J, HO WG, ET AL. Treatment of acute myelogenous leukemia: bone marrow transplantation versus consolidation chemotherapy. Proc ASCO 2:180, 1983.
97. THOMAS ED, CLIFT RA, and BUCKNER CE. Marrow transplantation for patients with acute nonlymphoblastic leukemia who achieve a first remission. Cancer Treat Rep 66:1463-1466, 1982.
98. POWLES RL, CLINK HM, BANDINI G, ET AL. The place of bone marrow transplantation in acute myelogenous leukaemia. Lancet 1:1047-1050, 1980.
99. KERSEY J, RAMSAY N, KIM T, ET AL. Allogeneic bone marrow transplantation (BMT) in acute non-lymphocytic leukemia: a pilot

study. Blood 60:400-403, 1982.

100. ARMITAGE J, KLASSEN L, KUGLER J, ET AL. Allogeneic bone marrow transplantation (BMT) for the treatment of acute leukemia. Clin Res 29:842A, 1981.

101. ZANDER AR, KEATING M, KANOJIA M, ET AL. Allogeneic bone marrow transplantation in acute leukemia: piperazinedione and fractionated total body irradiation as an alternative conditioning regimen. Transplant Proc 15:1392-1394, 1983.

102. MANNONI P, VERNANT JP, RODET M, ET AL. Marrow transplantation for acute non-lymphoblastic leukemia in first remission. Blut 41:220-225, 1980.

103. DINSMORE R, KIRKPATRICK D, FLOMENBERG N, ET AL. Allogeneic bone marrow transplantation for acute non-lymphocytic leukemia. Blood 60(suppl):167a, 1982.

104. SPECK B, GRATWOHL A, NISSEN C, ET AL. Further experience with cyclosporin A in allogeneic bone marrow transplantation. Exp Hematol 9(suppl 9):124, 1981.

105. GALE RP, KAY HEM, RIMM AA, ET AL. Bone marrow transplantation for acute leukemia in first remission: report of the International Bone Marrow Transplant Registry. Lancet 2:1006-1008, 1982.

106. PREISLER HD, RUSTUM Y, HENDERSON ES, ET AL. Treatment of acute non-lymphocytic leukemia: use of anthracycline—cytosine arabinoside induction therapy and comparison of two maintenance regimens. Blood 53:455-464, 1979.

107. WEINSTEIN HJ, MAYER RJ, ROSENTHAL DS, ET AL. Treatment of acute myelogenous leukemia. N Engl J Med 303:473-478, 1982.

108. FOON KA, and GALE RP. Controversies in the treatment of acute myelogenous leukemia. Am J Med 72:963-979, 1982.

109. WOLFF SN, MARION J, STEIN R, ET AL. Therapy of acute non-lymphocytic leukemia in first remission: brief intensive consolidation with high dose ara-C. Blood 60:159a, 1982.

110. KEATING MJ, SMITH TL, GEHAN EA, ET AL. Factors related to length of complete remission in adult acute leukemia. Cancer 45:2017-2029, 1980.

111. PREISLER HD, REESE PA, MARINELLO MJ, ET AL. Adverse effects of aneuploidy in the outcome of remission induction therapy for acute non-lymphocytic leukemia: analysis of types of treatment failure. Br J Haematol 53:459-466, 1983.

112. GOLOMB HM, VARDIMAN J, and ROWLEY JD. Correlation of clinical findings with quinacrine banded chromosomes in 90 adults with acute non-lymphocytic leukemia. N Engl J Med 299:613-619, 1978.

113. DINSMORE R, SHANK B, KAPOOR N, ET AL. A randomized trial of marrow transplantation (BMT) versus chemotherapy (CT) maintenance for acute myelogenous leukemia in first remission. Preliminary results. Exp Hematol 9(suppl 9):213, 1981.

114. APPELBAUM FR, CHEEVER MA, FEFER A, ET AL. A prospective study of the value of maintenance therapy or bone marrow transplantation in acute non-lymphoblastic leukemia. Blood 60:163a, 1982.

115. GYGER M, PERRCAULT C, BOILEAU J, ET AL. Restoration of normal hematopoiesis by bone marrow ablation and allogeneic marrow transplantation in a case of Hodgkin's disease therapy-related leukemia. Blood 61:1275-1277, 1983.

116. STORB R, SANDERS JE, RAMBERG R, ET AL. Marrow transplantation for treatment of preleukemia. Exp Hematol 7(supp 14):133, 1983.

117. ZANDER AR, DICKE KA, VELLEKOOP L, ET AL. Autografting in acute leukemia. *In* Recent Advances in Bone Marrow Transplantation (Gale RP, ed). New York, Alan Liss, 1983, pp 659-678.

118. HERZIG GP, PHILLIPS GL, MILL W, ET AL. Treatment of hematological malignancy with cyclophosphamide, total body irradiation and autologous bone marrow transplantation. Blood 52(supp 1):253, 1978.

119. GORIN NC, DAVID R, STACHOWIAK J, ET AL. High dose chemotherapy and autologous bone marrow transplantation in acute leukemias, malignant lymphomas and solid tumors; a study of 23 patients. Eur J Cancer 17:557-568, 1981.

120. ZANDER AR, VELLEKOOP L, SPITZER G, ET AL. Combination of high-dose cyclophosphamide, BCNU, and VP-16-213 followed by autologous marrow rescue in the treatment of relapsed leukemia. Cancer Treat Rep 65:377-381, 1981.

121. HERVE P, ROZENBAUM A, PLOUVIER E, ET AL. Autologous bone marrow transplantation in acute myeloid leukemia in relapse or complete remission. Cancer Treat Rep 66:1983-1985, 1982.

122. STEWART P, BUCKNER CD, and THOMAS ED. Autologous marrow transplantation in patients with acute non-lymphocytic leukemia in remission. J Cell Biochem Suppl 7A:57, 1983.

123. CHAMPLIN R, FEIG SA, STORCH S, ET AL. Bone marrow transplantation for acute lymphoblastic leukemia in remission. Proc Am Assoc Cancer Res 24:119, 1983.

124. DINSMORE R, KIRKPATRICK D, FLOMENBERG N, ET AL. Allogeneic marrow transplantation for acute lymphoblastic leukemia in remission: the importance of early transplantation. Transplant Proc 15:1397-1400, 1983.

125. WOODS WG, NESBIT ME, RAMSAY NKC, ET AL. Intensive therapy followed by bone marrow transplantation for patients with acute lymphocytic leukemia in second or subsequent remission: determination of prognostic factors. Blood 61:1182-1189, 1983.

126. CLIFT RA, BUCKNER CD, THOMAS ED, ET AL. Allogeneic marrow transplantation for acute lymphoblastic leukemia in remission using fractionated total body irradiation. Leukemia Res 6:409-412, 1982.

127. BARRETT AJ, KENDRA JR, LUCAS CF, ET AL. Bone marrow transplantation for acute lymphoblastic leukemia. Br J Haematol 52:181-188, 1982.

128. SCOTT EP, FORMAN SJ, SPRUCE WE, ET AL. Bone marrow ablation followed by allogeneic bone marrow transplantation for patients with high risk acute lymphoblastic leukemia during complete remission. Transplant Proc 15:1395-1396, 1983.

129. KENDRA JR, BARRETT AJ, LUCAS C, ET AL. Bone marrow transplantation for acute lymphoblastic leukemia. Exp Hematol 10(supp 10):72-73, 1982.

130. BLACKLOCK HA, MATTHEWS JRD, BUCHANAN JG, ET AL. Improved survival from acute lymphoblastic leukemia in adolescents and adults. Cancer 48:1931-1935, 1981.

131. ARLIN ZA, GEE TS, MERLELSMANN R, ET AL. Treatment of acute lymphoblastic leukemia in adults: comparability of results using extended and brief consolidation therapy. Proc Am Assoc Cancer Res 24:119, 1983.

132. GREEN DM, BRECHER ML, BLUMENSON LE, ET AL. The use of intermediate dose methotrexate in increased risk childhood acute lymphoblastic leukemia. Cancer 50:2722-2727, 1982.

133. RITZ J, PESANDO JM, NOTIS MCCONARTY J, ET AL. A monoclonal antibody to human acute lymphoblastic leukemia antigen. Nature 283:583, 1980.

134. GREAVES MF. Analysis of the clinical and biological significance of lymphoid phenotypes in acute leukemia. Cancer Res 41:4752-4766, 1981.

135. RITZ J, SALLAN SE, BAST RC, ET AL. Autologous bone marrow transplantation in CALLA positive acute lymphoblastic leukemia after in vitro treatment with J5 monoclonal antibody and complement. Lancet 2:60-63, 1982.

136. CHAMPLIN RE, TERRITO MC, HO WG, ET AL. Prolonged remission following autologous bone marrow transplantation for acute lymphoblastic leukemia. Blood 60(supp 1):165a, 1982.

137. KAISER H, LEVY R, and COTE JP. Autologous bone marrow transplantation in lymphoblastic lymphoma and T cell leukemia. Blood 60(supp 1):169a, 1982.

138. NETZEL B, RODT H, HAAS RJ, ET AL. Immunological conditioning of bone marrow for autotransplantation in childhood acute lymphoblastic leukemia. Lancet 2:1330-1332, 1980.

139. BAST RC, DEFABRITIS P, MAYER C, ET AL. Elimination of malignant clonogenic cells from human bone marrow using multiple monoclonal antibodies and complement. Proc ASCO 24:223, 1983.

140. VITETTA ES, KROLICK KA, MIYAMA-INABA M, ET AL. Immunotoxins: a new approach to cancer therapy. Science 219:644-650, 1983.

141. KOEFFLER HP, and GOLDE DW. Chronic myelogenous leukemia,

new concepts. N Engl J Med 304:1201-1209, 1269-1274, 1981.
142. SOKAL JE. Evaluation of survival data for chronic myelocytic leukemia. Am J Hematol 1:493-500, 1976.
143. COLEMAN M, SILVER R, PAJOK T, ET AL. Combination chemotherapy for terminal phase of chronic myelocytic leukemia. Blood 55:29-36, 1980.
144. GOTO T, NISHIKORI M, ARLIN Z, ET AL. Growth characteristics of leukemic and normal hematopoietic cells in Ph+ chronic myelogenous leukemia and effects of intensive treatment. Blood 59:793-808, 1982.
145. CHAMPLIN RE, MITSUYASU R, and GALE RP. The role of bone marrow transplantation in the treatment of chronic myelogenous leukemia. *In* Recent Advances in Bone Marrow Transplantation (Gale RP, ed). New York, Alan Liss, 1983, pp 141-158,
146. GOLDMAN JM, TH'NG KH, PARK DS, ET AL. Collection, cryopreservation and subsequent viability of hematopoietic cells intended for treatment of chronic granulocytic leukemia in blast cell transformation. Br J Haematol 40:185-195, 1978.
147. BUCKNER CD, STEWART P, CLIFT RA, ET AL. Treatment of blastic transformation of chronic granulocytic leukemia by chemotherapy, total body irradiation and infusion of cryopreserved autologous marrow. Exp Hematol 6:96-109, 1978.
148. GOLDMAN JM, CATOVSKY D, GOALDEN AW, ET AL. Buffy coat autografts for patients with chronic granulocytic leukemia in transformation. Blut 42:149-155, 1981.
149. FEFER A, CHEEVER MA, and THOMAS ED. Disappearance of Ph^1 positive cells in 4 patients with chronic granulocytic leukemia after chemotherapy, irradiation, and marrow transplantation from an identical twin. N Engl J Med 300:333-337, 1979.
150. FEFER A, CHEEVER MA, GREENBERG PD, ET AL. Treatment of chronic granulocytic leukemia with chemoradiotherapy and transplantation of marrow from identical twins. N Engl J Med 306:63-68, 1982.
151. GOLDMAN JM, JOHNSON SA, CATOVSKY D, ET AL. Identical twin marrow transplantation for patients with leukemia and lymphoma. Transplant 31:140-141, 1981.
152. SANTOS GW, BIAS W, BURNS W, ET AL. Syngeneic bone marrow transplantation, Baltimore experience. Exp Hematol 9(supp 9):141, 1981.
153. DONEY K, BUCKNER CD, SALE GE, ET AL. Treatment of chronic granulocytic leukemia by chemotherapy, total body irradiation and allogeneic bone marrow transplantation. Exp Hematol 6:738-747, 1978.
154. DONEY KC, BUCKNER CD, and THOMAS ED. Allogeneic bone marrow transplantation for chronic granulocytic leukemia. Exp Hematol 9:966-971, 1981.
155. MCGLAVE PB, MILLER WJ, HURD DD, ET AL. Cytogenetic conversion following allogeneic BMT for advanced chronic myelogenous leukemia. Blood 58:1050-1052, 1981.
156. CHAMPLIN R, HO W, ARENSON E, ET AL. Allogeneic bone marrow transplantation in chronic myelogenous leukemia in chronic or accelerated phase. Blood 60:1038-1041, 1982.
157. MESSNER HA, CURTIS JE, and NORMAL C. Allogeneic bone marrow transplantation in patients with CML prior to blast crisis. Blood 58(supp 1):175a, 1981.
158. MCGLAVE PB, KIM TH, HURD DD, ET AL. Successful allogeneic bone marrow transplantation for patients in the accelerated phase of chronic granulocytic leukemia. Lancet 2:625-627, 1982.
159. CLIFT RA, THOMAS ED, FEFER A, ET AL. Treatment of chronic granulocytic leukemia in chronic phase by allogeneic marrow transplantation. Lancet 2:621-622, 1982.
160. SPECK B, GRATWOHL A, NISSEN C, ET AL. Allogeneic marrow transplantation for chronic granulocytic leukemia. Blut 45:237-242, 1982.
161. GOLDMAN JM, MCCARTHY DM, HOWS JM, ET AL. Marrow transplantation for patients in chronic phase of chronic granulocytic leukemia. Lancet 2:623-625, 1982.
162. VERNANT JP, RODET M, ROCHANT H, ET AL. Successful allogeneic bone marrow transplantation after reversion to chronic phase of blast crisis in chronic myeloid leukemia. Am J Hematol 11:205-207, 1981.
163. TURA S, BACCARNI M, CORBELLI G, ET AL. Staging of chronic myeloid leukemia. Br J Hematol 47:105-119, 1981.
164. CERVANTES F, and ROZMAN C. A multivariate analysis of prognostic factors in chronic myeloid leukemia. Blood 60:1298-1304, 1981.
165. CLIFT RA, HANSEN JA, THOMAS ED, ET AL. Marrow transplantation from donors other than HLA identical siblings. Transplantation 28:235-242, 1979.
166. POWLES RL, KAY HEM, CLINK HM, ET AL. Mismatched family donors for bone marrow transplantation as treatment for acute leukemia. Lancet 1:612-615, 1983.
167. HANSEN JA, CLIFT RA, THOMAS ED, ET AL. Transplantation of marrow from an unrelated donor to a patient with acute leukemia. N Engl J Med 303:565-567, 1980.
168. OPELZ G, MICKEY MR, and TERASAKI PI. Unrelated donors for bone marrow transplantation and transfusion support: pool sizes required. Transplant Proc 6:405-409, 1974.
169. PHILLIPS GL. Current clinical trials with intensive therapy and autologous bone marrow transplantation for lymphomas and solid tumors. *In* Recent Advances in Bone Marrow Transplantation (Gale RP, ed). New York, Alan Liss, 1983, pp 567-597.
170. SPITZER G, ZANDER A, and TANNIR N. Autologous bone marrow transplantation in human solid tumors. *In* Recent Advances in Bone Marrow Transplantation (Gale RP, ed). New York, Alan Liss, 1983, pp 615-642.
171. APPELBAUM FR, FEFER A, CHEEVER MA, ET AL. Treatment of non Hodgkin's lymphoma with marrow transplantation in identical twins. Blood 58:509-513, 1981.
172. DOUER D, CHAMPLIN RE, HO WG, ET AL. High dose combined modality therapy and autologous bone marrow transplantation in resistant cancer. Am J Med 71:973-976, 1981.
173. GOLDMAN JM, JOHNSON SA, CATOVSKY D, ET AL. Identical twin transplantation for patients with leukemia and lymphoma. Transplantation 31:140-141, 1981.
174. SANTOS GW, BIAS W, BURNS W, ET AL. Syngeneic bone marrow transplantation; the Baltimore Experience. Exp Hematol 9(supp 9):141, 1981.
175. O'LEARY M, RAMSAY NKC, NESBIT ME, ET AL. Bone marrow transplantation for non Hodgkin's lymphoma in children and young adults. Am J Med 74:497-501, 1983.
176. APPELBAUM FR, DEISSEROTH AB, GRAW RG, ET AL. Prolonged complete remission following high dose chemotherapy of Burkitt's lymphoma in relapse. Cancer 41:1059-1068, 1978.
177. GORIN NC, DAVID R, STRACHOWIAK J, ET AL. High dose chemotherapy and autologous bone marrow transplantation in acute leukemias, malignant lymphomas and solid tumors. Eur J Cancer 17:557-568, 1981.
178. BENSINGER W, BUCKNER CD, STEWART P, ET AL. Autologous bone marrow transplantation for end stage lymphoma. J Cell Biochem Suppl 7A:58, 1983.
179. PHILLIPS G, HERZIG G, HERZIG R, ET AL. Cyclophosphamide, total body irradiation and autologous bone marrow transplantation for refractory malignant lymphoma. Blood 58(supp 1):175a, 1981.
180. SEEGER RC, SIEGAL SE, and SIDELL H. Neuroblastoma: clinical prospectives monoclonal antibodies and retinoic acid. Ann Intern Med 97:873, 1982.
181. GRAHAM POLE J, GROSS S, HERZIG R, ET AL. High dose melphalan (L-PAM) and bone marrow autografting for refractory neuroblastoma and Ewing's sarcoma. Clin Res 30:70, 1982.
182. PRITCHARD J, MCELWAIN TJ, and GRAHAM POLE J. High dose melphalan with autologous marrow transplantation for treatment of advanced neuroblastoma. Br J Cancer 45:86-94, 1981.
183. AUGUST CS, SEROTA FT, KOCH PA, ET AL. Transplantation for relapsed stage IV neuroblastoma. Proc Am Assoc Cancer Res 23:142, 1982.
184. CORNBLEET MA, CORRINGHAM REJ, PRENTICE HG, ET AL. Treatment of Ewing's sarcoma with high-dose melphalan and autologous bone marrow transplantation. Cancer Treat Rep 65:241-244, 1981.

185. TEPPER J, GLAUBIGER, LICHTER A, ET AL. Local control of Ewing's sarcoma of bone with radiotherapy and combination chemotherapy. Cancer 46:1969-1973, 1980.

186. LAZARUS HM, HERZIG RH, GRAHAM POLE J, ET AL. Intensive melphalan chemotherapy and cryopreserved autologous bone marrow transplantation for the treatment of refractory cancer. J Clin Oncol 1:359-367, 1983.

187. BLIJHAM G, SPITZER A, and LITAM J. The treatment of advanced testicular carcinoma with high dose chemotherapy and autologous marrow support. Eur J Cancer 17:443, 1981.

188. SPITZER G, DICKE KA, LITAM J, ET AL. High dose combination chemotherapy with autologous bone marrow transplantation in adult solid tumors. Cancer 45:3075-3085, 1980.

189. FAHRA P, SPITZER G, and VALDIVIESO M. High dose intensification with autologous bone marrow transplantation in limited disease small cell lung cancer (SCLC). J Cell Biochem Suppl 7A:63, 1983.

190. WEIDEN PL, STORB R, DEEG J, ET AL. Prolonged disease free survival in dogs with lymphoma after total body irradiation and autologous marrow transplantation: consolidation of combination chemotherapy induced remissions. Blood 54:1039-1049, 1979.

191. PHILLIPS GL, FAY JW, HERZIG GP, ET AL. Intensive 1, 3-bis (2 chloroethyl)-1-nitrosourea (BCNU) autologous bone marrow transplantation therapy of refractory cancer: a preliminary report. Exp Hematol (suppl 5)7:372, 1979.

192. STEWART PS. Autologous bone marrow transplantation in metastatic breast cancer. Breast Cancer Res Treat 2:85, 1982.

193. SARNA GP, CHAMPLIN R, WELLS J, ET AL. Phase I study of high-dose mitomycin with autologous bone marrow support. Cancer Treat Rep 66:277-282, 1982.

194. HOCHBERG FH, PARKER LM, TAKVORIAN T, ET AL. High dose BCNU with autologous bone marrow rescue for recurrent glioblastoma multiforme. J Neurosurg 54:455, 1981.

195. BUCKNER CD, RUDOLF RH, FEFER A, ET AL. High dose cyclophosphamide therapy for malignant disease. Cancer 29:357-365, 1972.

196. WOLFF S, FER MF, MACKAY C, ET AL. High dose VP-16 and autologous bone marrow transplantation. Proc Am Assoc Cancer Res 23:134, 1982.

197. ZANDER AR, SPITZER G, LEGHA S, ET AL. High-dose AMSA and bone marrow rescue in patients with solid tumors. Cancer Treat Rep 66:385-386, 1982.

198. BUCKNER CD, STEWART PS, BENSINGER W, ET AL. Critical issues in autologous marrow transplantation for hematologic malignancies. *In* Recent Advances in Bone Marrow Transplantation (Gale RP, ed). New York, Alan Liss, 1983, pp 599-613.

Antiemetic Therapy: A Review of Recent Studies and a Report of a Random Assignment Trial Comparing Metoclopramide With Delta-9-tetrahydrocannabinol[1,2]

Richard J. Gralla,* Leslie B. Tyson, Laurie A. Bordin, Rebecca A. Clark, David P. Kelsen, Mark G. Kris, Leonard B. Kalman, and Susan Groshen[3]

The treatment of emesis in patients receiving chemotherapeutic agents has been the subject of increased investigation over the past 5 years. Studies have examined several classes of antiemetics and have identified several drugs with encouraging activity. The more successful trials have concentrated on agents, doses, schedules, or routes of administration that were not generally used prior to 1980.

The problem of emesis associated with chemotherapy has been clear for many years, but has become more pressing as the role of chemotherapy has enlarged. Although many questions about antiemetic treatment remain, recent trials have increased the understanding of the problem of emesis, helped define effective study design, and indicated several promising directions for further investigation.

The purpose of this paper is twofold. First, we report the results of a recently completed study in which two frequently used agents, metoclopramide and delta-9-tetrahydrocannabinol (THC), were compared. Second, we review recent trials with several antiemetic agents and examine trends observed in current antiemetic clinical research.

I. High-Dose Metoclopramide Versus THC: A Random Assignment Trial in Patients Receiving Cisplatin

Among the newer antiemetic agents undergoing clinical trials, the substituted benzamide metoclopramide and the cannabinoid THC have been reported to have encouraging activity. Both agents have been tested in double-blind trials and have been observed to be superior to placebo and phenothiazine prochlorperazine (PCP) (1-5). Additionally, open studies have reported useful antiemetic results with metoclopramide (6-8) and THC (9,10).

To date, trials have indicated a dose-response relationship with metoclopramide. Earlier studies using this agent in low oral doses (20 mg) did not observe consistent antiemetic efficacy against cisplatin (11). However, a phase I trial demonstrated that metoclopramide could be administered safely in high iv doses (12) and further reports indicated that these doses (1-2 mg/kg) were effective (4,5). Recently, a pharmacokinetic study correlated high blood levels of metoclopramide ($>$ 840 ng/ml) with efficacy (13). The dose and schedule of metoclopramide used in the present trial were selected due to observed activity in previous studies in patients receiving high doses of cisplatin (4,7).

Although THC has been studied for several years, no consensus has been reached on the best dose or scheduling of this agent (14). The majority of trials have investigated doses of 2.5 mg-10 mg/m^2 given orally every 3-4 hours, beginning at variable times prior to chemotherapy. THC, at a dose of 10 mg/m^2 every 3 hours, was selected for the present trial because of reported efficacy with a similar regimen by Chang et al (1).

This study was conducted to compare the efficacy of these two useful antiemetics in patients receiving a chemotherapeutic agent, cisplatin, that produces emesis in nearly all patients (4,5).

PATIENTS AND METHODS

Thirty-one patients with histologically confirmed malignancy were entered in this trial. Patients were required to have a performance status $>$ 50% (Karnofsky scale) and to be receiving their first course of cisplatin at a dose of 120 mg/m^2 iv. As required for the chemotherapy protocols, patients had a wbc count $\geqslant$ 4000 cells/mm^3, a platelet count $\geqslant$ 120,000/mm^3, a creatinine clearance $\geqslant$ 65 ml/minute, and a serum bilirubin $<$ 2.0 mg/dl. Informed consent was obtained from all patients. Charac-

---

[1] Supported in part by a grant from the A. H. Robins Co, and by Public Health Service grant CA-05826 and contract N01-CM-07337 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[2] Presented in part at the Eighteenth Annual Meeting of the American Society of Clinical Oncology, Apr 1982.

[3] Developmental Chemotherapy and Solid Tumor Services, Department of Medicine and the Biostatistics Laboratory, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY.

* *Reprint requests to:* Dr. Richard J. Gralla, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

teristics of the 30 adequately treated patients are given in table 1.

Patients were randomly assigned to receive either metoclopramide or THC. Metoclopramide (Reglan, A. H. Robins, Richmond, VA) was supplied in 50- and 2-ml vials, containing 150 and 10 mg of the agent, respectively. Metoclopramide (2 mg/kg) or an identical volume of placebo (for patients receiving THC) was added to 50 ml of 0.9% sodium chloride and infused over 15 minutes at the time of each dose. The dosage was kept constant throughout each trial and was administered at the following times: 30 minutes prior to cisplatin and 1½, 3½, 5½, and 8½ hours after therapy (the total dose of metoclopramide was 10 mg/kg during the study period).

THC was supplied in 5- and 2.5-mg capsules (National Cancer Institute, Bethesda, MD). Patients were given either THC at a dose of 10 mg/m^2 orally or an identical number of placebo-filled capsules (for patients assigned to receive metoclopramide). THC was given 1½ hours before cisplatin and 1½, 4½, 7½, and 10½ hours after chemotherapy (total dose of 50 mg/m^2 of THC during the study period).

This study used a paired design, in which one patient in every pair was randomly assigned to each treatment. The double-blind design was maintained by giving all patients both an iv and an oral dose at the times specified above, by the use of placebo in addition to the active agents. Food or fluids by mouth were not allowed during the initial 12 hours of the trial; no sedative or other antiemetic drugs were given 10 hours before or during the study. Patients who had antiemetic benefit with any agent were offered that agent during subsequent chemotherapy.

The number of episodes and the volume of emesis and the duration of nausea and vomiting were recorded for each patient. All patients were observed in the hospital; the period of observation included the 24 hours after cisplatin administration. Side effects of treatment were recorded, which included sedation, number of bowel movements, and dystonic reactions. Sedation was graded as follows: none, mild (patient lethargic but aroused by verbal stimuli and completely oriented when awakened), moderate (patient aroused only by physical stimuli and completely oriented when awakened), and marked (patient aroused only by physical stimuli and disoriented when awakened). In addition, the presence or absence of a "high" was recorded, as was evidence of orthostatic hypotension (decrease ⩾ 20 mm Hg), dizziness, or dry mouth.

All patients in this study were hospitalized to receive cisplatin at a dose of 120 mg/m^2 iv in a 20-minute infusion. The cisplatin was given with vigorous hydration and mannitol, according to the method of Hayes et al (15) and as previously outlined (4). Patients with lung or esophageal cancers also received a vinca alkaloid (vindesine or vinblastine) during the treatment period; these agents generally do not induce emesis (16).

RESULTS

Of the 31 patients entered in this trial, 30 completed the course of chemotherapy with antiemetics and were evaluable for antiemetic response. One patient with lung cancer and a history of atherosclerotic cardiovascular disease experienced the onset of atrial fibrillation 1 hour after receiving cisplatin. The patient was taken off study to determine which antiemetic he had received, in case this factor might influence further management; the arrhythmia resolved spontaneously. This patient had been randomly assigned to receive metoclopramide and had been given only the initial dose. He received no further antiemetics and experienced no emesis, and had no other evidence of cardiovascular problems.

As shown in table 2, patients receiving metoclopramide had significantly fewer episodes of vomiting (median: two vs eight) than those receiving THC ($P = 0.02$). Additionally, more of the patients who received metoclopra-

TABLE 1.—Characteristics of patients receiving high-dose metoclopramide or THC*

Characteristic	Metoclopramide	THC
Total No. of patients	15	15
Age in yrs		
Median	58	58
Range	45-70	39-72
Sex		
Men	10	13
Women	5	2
Performance status		
80%-90%	8	10
60%-70%	7	5
Diagnosis		
Bronchogenic carcinoma	12	12
Esophageal carcinoma	2	2
Head and neck carcinoma	1	1

*Unless otherwise specified, values = No. of patients.

TABLE 2.—Antiemetic results of high-dose metoclopramide vs THC in patients receiving cisplatin

Characteristic	Metoclopramide	THC	*P* value
Total No. of patients	15	15	
No. of emetic episodes			
Median	2	8	0.02
Range	0–14	0–18	
% of patients with major antiemetic response (0–2 episodes)	73%	27%	0.01
Volume of emesis (ml)			
Median	30	275	0.09
Range	0–465	0–760	
Median duration in hrs of			
Vomiting	0.33	2.17	0.06
Nausea	0.33	0.83	1.0

mide (73% vs 27%, $P = 0.01$) had major antiemetic control (defined as two or fewer vomiting episodes). The volume of emesis and the durations of nausea and vomiting were less for patients receiving metoclopramide; however, these differences were not significant ($P < 0.05$) in the small number of patients entered in this study.

Although metoclopramide was the superior agent in this trial, it should be noted that useful antiemetic activity was observed with THC. In prior studies at our institution with similar groups of patients receiving cisplatin either with placebo or im PCP (4), none of 20 patients had major antiemetic control (27% with THC in the present trial).

Both metoclopramide and THC were generally well-tolerated by the patients (table 3). Overall, side effects were more common in patients receiving THC. Sedation occurred with both agents and was usually mild, with a tendency toward a greater degree of sedation in patients given THC. Autonomic reactions, such as orthostatic hypotension or dry mouth, were observed in nearly all patients receiving THC and were uncommon in patients assigned to metoclopramide.

Three patients receiving THC (20%) and one given metoclopramide reported experiencing a "high," defined as a mood alteration with euphoria or dysphoria, or the occurrence of hallucinations. In no instance was it necessary to stop antiemetic treatment because of this effect. There did not appear to be any relationship between the occurrence of a "high" and antiemetic response in this small number of patients receiving THC, who also experienced pharmacologic evidence of the cannabinoids in the form of the observed autonomic reactions.

Dystonic reactions were not observed in patients on either arm of this trial. Diarrhea (three or more loose bowel movements in the 24-hour observation period) was seen only in patients receiving metoclopramide. Because cisplatin itself has been reported to cause diarrhea (18), the absence of this effect may be due to antidiarrheal properties of the cannabinoids.

Other toxic effects from the chemotherapeutic agents, such as myelosuppression, nephrotoxicity, or peripheral neuropathy, did not appear to be altered by either of the antiemetic agents. Additionally, antitumor activity was similar on both arms of the study and was not changed from that previously observed with these chemotherapeutic regimens.

TABLE 3.—Toxic effects in 30 patients receiving high-dose metoclopramide or THC

Toxic effect	Metoclopramide ($N = 15$)	THC ($N = 15$)
Sedation		
Mild	93%	73%
Moderate	0%	13%
Orthostatic hypotension	0%	53%
Dizziness	7%	80%
Dry mouth	33%	80%
"High"	7%	20%
Dystonic reactions	0%	0%
Median No. of bowel movements (per patient over 24 hrs)	3	0

DISCUSSION

The results of this trial indicate that metoclopramide is an effective antiemetic agent and is superior to THC in patients receiving cisplatin. The high iv doses of metoclopramide were well-tolerated in this population of patients and did not alter the efficacy of the chemotherapeutic agents used.

Some antiemetic activity was observed with THC, although side effects were generally more frequent with this agent. It is possible that lower doses of THC would be associated with decreased toxicity; however, a further lessening of efficacy would be a potential disadvantage. Some prior studies have indicated better activity with THC in patients receiving chemotherapeutic agents that are less likely to produce emesis than cisplatin (1,9,19).

Dystonic reactions, previously reported with metoclopramide (4,11), were not observed in this trial. An analysis of nearly 500 patients receiving metoclopramide at our institution has shown a strong correlation between age (< 30 years) and likelihood of developing trismus or torticollis. Only 1.8% of patients ⩾ 30 years of age had dystonic reactions, as opposed to a 27% incidence in younger patients ($P = 0.000015$) (20). It is possible that other schedules of metoclopramide administration might alter the incidence of these reactions. In general, dystonic reactions clear rapidly following iv diphenhydramine (20).

Recent reports have indicated that metoclopramide has antiemetic effectiveness against a broader range of chemotherapeutic drugs than just cisplatin. In open trials, Strum et al (6) have noted efficacy against the emesis produced by alkylating agents and anthracyclines, while Tyson et al (21) have reported useful activity for patients receiving dacarbazine. Further trials will be required to determine the best schedule of administration of metoclopramide, and whether doses as high as those used in the current study in patients receiving cisplatin are necessary for patients given other chemotherapeutic drugs.

This study demonstrates that metoclopramide is superior to THC in decreasing emesis induced by cisplatin and supports previous trials reporting the antiemetic efficacy of metoclopramide (4-8).

II. Review of Recent Antiemetic Studies

The number of antiemetic studies in patients receiving cancer chemotherapy has increased markedly over the past several years (22). These reports have varied from observations of a few patients to formal phase I and phase II trials and, more recently, to random assignment double-blind studies with drug doses and schedules based on the results of open trials.

Table 4 lists a number of chemotherapeutic agents by their likelihood of causing emesis. Differences occur among patients and even among treatment courses in the same patient; any of the drugs in this table can produce emesis in an individual patient.

TABLE 4.—Emetic potential of 20 commonly used chemotherapeutic agents

	Chemotherapeutic agent
High incidence of emesis	Cisplatin
	Dacarbazine
	Dactinomycin
	Mechlorethamine
	Streptozocin
	Cyclophosphamide
	Carmustine
	Lomustine
	Doxorubicin
	Daunorubicin
	Procarbazine
	Mitomycin
	Cytarabine
	Etoposide
	Methotrexate
	5-FU
	Vinblastine
	Vincristine
	Bleomycin
Low incidence of emesis	Chlorambucil

In most instances, both the drug dose and the route of administration are important variables than can affect the incidence of nausea and vomiting. For most of the drugs, the onset of emesis occurs between 1 and 2 hours after chemotherapy in previously untreated patients. An exception to this is observed with cyclophosphamide; with this agent, the emesis may be delayed until ⩾ 12 hours after drug administration (23).

The physiology and neuropharmacology of chemotherapy-induced emesis are still not completely understood (24). Reflex-induced vomiting may be caused by irritation of the gastrointestinal tract or stimulation of receptors in the central nervous system. Receptor areas have been identified that affect a common vomiting center located in the lateral reticular formation of the medulla, which then coordinates the act of vomiting. An important area also located in the medulla is the chemoreceptor trigger zone (CTZ) in the floor of the fourth ventricle in the area postrema. This receptor region is sensitive to chemical stimuli from both the blood and the cerebrospinal fluid. The hypothesis that neurotransmitter receptors are found in the CTZ is important in therapeutic considerations. Thus, chemotherapeutic agents or their metabolites may, in some manner, stimulate receptors, such as dopamine receptors, in the CTZ. Impulses generated in the CTZ and transmitted to the vomiting center may then lead to the initiation of emesis. Neuroreceptors in the gastrointestinal tract with afferents to the vomiting center may also play a role in the emesis associated with chemotherapy. A third area of importance is the cortex of the brain. The familiar problem of vomiting in anticipation of chemotherapy is clearly not associated with exogenous chemical stimulation, but would appear to be mediated initially by the cortex which may then stimulate the medullary vomiting center. Antiemetic agents that block neuroreceptors in the CTZ or in the gastrointestinal tract may limit the emetic effect of chemotherapeutic drugs. Effective agents may not be active against all drug-induced emesis, or against anticipatory emesis, in that differing physiologic pathways may be involved.

Several different emetic problems have become apparent in patients receiving chemotherapy. These include: (*a*) acute chemotherapy-induced emesis; (*b*) anticipatory emesis; and (*c*) persistent or delayed emesis. A fourth problem is nausea or vomiting not induced by chemotherapy, but related instead to other medications that a patient is receiving, or to the tumor itself. In these instances, adjustment of medications or treatment of the complications of the tumor may be more important than the choice of the antiemetic agent.

The majority of studies have investigated agents in patients with acute chemotherapy-induced emesis, and this will be the primary focus of the present review. Anticipatory emesis, defined as nausea or vomiting starting before the administration of chemotherapy in a patient with past poor antiemetic control, remains a significant problem. The hospital environment and other treatment-related associations may trigger the emetic response. The administration of chemotherapy itself may also serve to bring on this response, not only as a chemical stimulus, but also as a psychologic factor. Anticipatory emesis appears to be more likely to develop in patients who are given chemotherapy of high emetic potential. Treatment that reduces the anxiety associated with further chemotherapy may be of value. If nausea and vomiting can be prevented in initial treatment cycles, then anticipatory emesis can be avoided. Factors related to the development of anticipatory emesis have recently been investigated by Morrow (25), who has also demonstrated the beneficial effect of behavior therapy for this problem (26).

Emesis may persist for several days and, in unusual instances, even for weeks. Some patients may achieve good antiemetic control while in the hospital, only to develop significant nausea after returning home. Other factors, such as metabolites of the chemotherapeutic agent, or even associations similar to those involved in anticipatory emesis, may play a role in the development of this problem. To date, no study has carefully examined the problem or treatment of delayed or persistent emesis.

The objective of this review is to outline studies that are representative of current trends in antiemetic treatment, rather than give an encyclopedic listing of all reports. While the studies have varied in quality and design, it has become clear that several factors are important when planning or evaluating an antiemetic study. These factors include: (*a*) standardization of the emetic stimulus; (*b*) accuracy in data collection, with the use of objective parameters when possible, such as the number of emetic episodes, and the volume and duration of emesis; (*c*) standardization of the patient population, with indication of whether or not patients had previously received chemotherapy; and (*d*) proper selection of route of administration, and drug schedule and dosage, based on prior trials with the agent. As with antitumor agents, it is important to determine quantitatively the efficacy of antiemetic agents used singly, so that results can be compared and further trials, including combination studies, can be planned appropriately.

Antiemetic Agents

Tables 5-8 outline the results of random assignment comparison antiemetic trials. The agents studied in these trials include metoclopramide, THC, dexamethasone,

TABLE 5.—Random assignment comparison trials of antiemetic agents with placebo

Antiemetic agent	Chemotherapeutic agent	Result	Ref No.
Metoclopramide (iv)			
Trial 1	Cisplatin	Metoclopramide superior to placebo	4
Trial 2	Cisplatin	Metoclopramide superior to placebo	5
THC			
Trial 1	Methotrexate	THC superior to placebo	1
Trial 2	Nitrosourea + 5-FU	THC superior to placebo	2
Trial 3	Various	THC superior to placebo	42
Dexamethasone			
Trial 1	Cyclophosphamide + methotrexate + 5-FU	Dexamethasone superior to placebo	50
PCP			
Trial 1	Nitrosourea + 5-FU	PCP superior to placebo	2
Trial 2	Various	PCP and placebo equivalent and both ineffective	42

TABLE 6.—Random assignment comparison trials of antiemetic agents with PCP

Antiemetic agent	Chemotherapeutic agent	Result	Ref No.
Metoclopramide (iv)			
Trial 1	Cisplatin	Metoclopramide superior to im PCP	4
Trial 2	Cisplatin	Metoclopramide equivalent to iv PCP	46
THC			
Trial 1	Nitrosourea + 5-FU	THC equivalent to PCP, but less toxicity with PCP	2
Trial 2	Various	THC superior to PCP	3
Trial 3	Various	THC superior to PCP	42
Placebo			
Trial 1	Nitrosourea + 5-FU	PCP superior to placebo	2
Trial 2	Various	PCP and placebo equivalent and both ineffective	42

PCP (prochlorperazine), and haloperidol. Comparisons with placebo are also given. These agents represent the major classes of drugs investigated in antiemetic trials. In many instances, open studies helped to identify useful activity and proper dosing prior to the comparison trials. Details concerning these agents are discussed below.

Metoclopramide

Metoclopramide is the first substituted benzamide to be widely used as an antiemetic. The agent probably exerts its antiemetic activity by blocking dopamine receptors in the CTZ and the upper gastrointestinal tract (27). Preclinical trials in dogs and ferrets indicated excellent activity against emesis produced by apomorphine (17), copper sulfate (17), and cisplatin (28,29), when metoclopramide was given in high doses. As mentioned in the first section of this paper, studies with low oral doses of metoclopramide (0.3 mg/kg) failed to show consistently useful activity with this agent (11); however, the agent was demonstrated to be safe for iv use in high doses (1-3 mg/kg) and was effective in initial open trials (12). Several phase II studies also reported efficacy with metoclopramide given in high iv doses (5-8). The importance of achieving high blood levels was underscored by a study demonstrating a correlation between antiemetic activity and the metoclopramide blood level (13). A preliminary study using high doses of metoclopramide orally has recently reported encouraging activity in a small number of patients (30).

As seen in tables 5-8, metoclopramide is one of the most widely studied agents in comparison trials. When given in high iv doses, metoclopramide has generally been shown to be superior, or at least equivalent, to all other antiemetic agents that it has been compared with in formal studies, against the emesis induced by cisplatin. Preliminary reports have indicated activity against emesis produced by several other chemotherapeutic

TABLE 7.—Random assignment comparison trials of antiemetic agents with THC

Antiemetic agent	Chemotherapeutic agent	Result	Ref No.
Metoclopramide (iv)			
Trial 1	Cisplatin	Metoclopramide superior to THC	This report
PCP			
Trial 1	Nitrosourea + 5-FU	PCP equivalent to THC, but less toxicity with PCP	2
Trial 2	Various	THC superior to PCP	3
Trial 3	Various	THC superior to PCP	42
Haloperidol			
Trial 1	Various	Haloperidol equivalent to THC, with less toxicity observed with haloperidol	55
Placebo			
Trial 1	Methotrexate	THC superior to placebo	1
Trial 2	Nitrosourea + 5-FU	THC superior to placebo	2
Trial 3	Various	THC superior to placebo	42

TABLE 8.—Random assignment comparison trials of antiemetic agents with iv metoclopramide

Antiemetic agent	Chemotherapeutic agent	Result	Ref No.
THC			
Trial 1	Cisplatin	Metoclopramide superior to THC	This report
Dexamethasone			
Trial 1	Cisplatin	Metoclopramide superior to dexamethasone, but both agents effective	51
Trial 2	Cisplatin	Metoclopramide (in high oral doses) equivalent to dexamethasone; both effective	52
PCP			
Trial 1	Cisplatin	Metoclopramide superior to im PCP	4
Trial 2	Cisplatin	Metoclopramide equivalent to iv PCP	46
Haloperidol			
Trial 1	Cisplatin	Metoclopramide superior to iv haloperidol, but both agents effective	56
Placebo			
Trial 1	Cisplatin	Metoclopramide superior to placebo	4
Trial 2	Cisplatin	Metoclopramide superior to placebo	5

agents (6,21,31). Metoclopramide has not been shown to be useful for patients with anticipatory emesis or vomiting refractory to other antiemetics.

The toxic effects commonly associated with metoclopramide have been mild sedation, dystonic reactions (which are age related), diarrhea (which may be associated with specific chemotherapeutic agents, eg, cisplatin, as well), and akathisia (4,6-8,18,20).

As outlined in the first section of this paper, both the dose and schedule of metoclopramide are important if antiemetic activity is to be observed. For patients receiving chemotherapeutic agents likely to produce emesis, current recommendations are for metoclopramide to be given at a dose of 1-2 mg/kg in a 15-minute infusion, repeated every 2 hours for three to five doses. The highest amounts are required for patients receiving cisplatin or dacarbazine in high doses. Current investigative regimens at several institutions include: (*a*) shorter courses of the antiemetic (two doses over 2 hours) in higher doses (2-3 mg/kg) for outpatient administration, and (*b*) the use of metoclopramide in combination with other antiemetics (32-34). The results of random assignment comparison trials with metoclopramide are summarized in table 8.

THC

Clinical trials with cannabinoids were encouraged by anecdotal reports of decreased emesis in younger patients who used marijuana when receiving their chemotherapy. The majority of trials have been conducted with delta-9-THC (35), although two synthetic cannabinoids, nabilone and levonantradol, have also been tested in several clinical studies (36-40). The mechanism of action of the cannabinoids remains unclear.

THC has been studied at a variety of doses using several schedules; however, in most trials, the doses have been 5 to 10 mg/m^2 every 3-4 hours. Antiemetic results have been conflicting in some studies (1,2,9,41); however, as outlined in table 7, THC has generally been found to be superior to placebo and equivalent or superior to oral PCP. Similar antiemetic findings have been reported with nabilone (36). THC appears to be more effective against chemotherapeutic agents of lower emetogenic potential (9,19,42).

Toxicity has been frequent, but generally manageable, with THC and other cannabinoids. The side effects have included sedation, dry mouth, orthostatic hypotension, ataxia, dizziness, and a "high." These reactions have caused a decreased acceptance of cannabinoids by some patients, especially those in older age groups (35). Conflicting results have been reported concerning whether or not a patient must experience a "high" to achieve good antiemetic control. The toxic effects observed with nabilone and levonantradol have been similar to those reported with THC.

Although study design has varied greatly among published trials with THC (14), most reviewers have concluded that this agent has antiemetic efficacy with acceptable toxicity at the doses listed above (22,35).

Prochlorperazine

PCP has been the most frequently used agent in the US for treatment of nausea and vomiting produced by chemotherapy. Structure-activity studies have indicated that variations of the side chain at position 10 of the phenothiazine ring affect the antiemetic properties of these agents (43). Phenothiazines with a piperazine group, such as PCP, have greater predicted antiemetic activity, but also lead to increased extrapyramidal reactions as opposed to those with aliphatic side chains (eg, chlorpromazine). This relationship has not been clearly established in patients with emesis caused by chemotherapy. Evidence, such as activity against apomorphine-induced emesis, indicates that phenothiazines exert their activity by blocking dopamine receptors in the CTZ (17,43). With the evidence currently available, it is difficult to recommend one phenothiazine above another. This review and table 6 examine PCP in particular, because this agent has been studied in comparison trials more than other phenothiazines.

PCP is available in a variety of administration forms. The parenteral route is useful for a patient who has difficulty taking oral agents because of emesis. A rectal suppository is more convenient than parenteral drug, but is less reliably absorbed than the oral form (44).

Random assignment comparative trials with PCP (table 6) have shown the following results in patients with chemotherapy-induced emesis: (*a*) superiority of oral PCP over placebo; (*b*) oral PCP inferior or equivalent to THC; and (*c*) im PCP inferior to high-dose metoclopramide in patients receiving cisplatin. In trials comparing PCP with THC, the phenothiazine generally produced less toxicity than the cannabinoid. Toxicity with oral PCP, usually sedation and, occasionally, extrapyramidal reactions (especially in younger patients), has been mild in the experience of most investigators.

Two reports have noted encouraging results with iv phenothiazines. In a preliminary report, good antiemetic control was achieved with both iv PCP and high-dose metoclopramide. Although iv phenothiazines can occasionally produce severe hypotension, this reaction was not noted in these reports. Further investigations directed at determining the most effective and safe method of phenothiazine use, especially with iv drug, appear to be indicated (45,46).

Dexamethasone

Several open trials have reported useful antiemetic results with corticosteroids (47-49). For the most part, studies have used either dexamethasone or methylprednisolone. Whether the mechanism of antiemetic activity for corticosteroids is based on a direct action of the drugs or is due to a feedback inhibition is unclear.

Formal random assignment comparison trials have been conducted only recently. These studies have used dexamethasone, and are outlined in tables 5 and 8. In patients receiving cyclophosphamide, methotrexate, and 5-FU combination chemotherapy, dexamethasone was found to be superior to placebo (50). Two studies in patients receiving cisplatin have compared dexamethasone with metoclopramide (51,52). In one trial, metoclopramide was superior to dexamethasone, but both agents were effective antiemetics (51). In the second study, in which metoclopramide was given orally in most doses, equivalent activity for both agents was observed, with a toxicity advantage reported for dexamethasone (52).

Doses of dexamethasone have generally ranged between 4 and 20 mg/administration, given orally or iv. Studies have administered corticosteroids in a single dose or have continued the agent every 4-6 hours for 1-2 days. Toxicity with these short-course regimens of steroids has been mild. Most investigators have reported occasional euphoria and rare instances of mild fluid retention, but in the majority of instances, no adverse reactions have occurred.

As confirmed in random assignment trials, dexamethasone is an effective antiemetic. With its low degree of toxicity, dexamethasone appears to be an excellent candidate for use in combination antiemetic studies.

Haloperidol

Both haloperidol and droperidol were reported to be effective antiemetics in initial trials (53,54). Like the phenothiazines and metoclopramide, the butyrophenones block dopamine receptors and probably exert their antiemetic activity in the CTZ of the area postrema. Random assignment studies have shown: (*a*) oral haloperidol to be equivalent to THC, as listed in table 7 (55); and (*b*) iv haloperidol to be effective in patients receiving cisplatin (table 8), although slightly less efficacious than high-dose metoclopramide (56). In oral and iv studies, doses have varied from 1 to 3 mg given every 2-6 hours. Toxic effects have been mild and similar to those seen with the phenothiazines, including sedation and extrapyramidal reactions.

Combination Studies

With the increased number of antiemetic studies over the last 4-5 years, including comparison trials, the relative activities and side effects of these agents have been outlined. Although several drugs have useful activity, none is ideal. The information gathered recently provides the single-agent knowledge necessary for designing combination regimens that are likely to improve efficacy.

The design of trials with combinations of antiemetics should focus on the following: (*a*) regimens should combine agents that do not have overlapping toxicity; (*b*) agents should not have opposing pharmacologic actions; (*c*) drugs should have demonstrated activity when used singly, and their doses, best route of administration, and schedules should be previously established; (*d*) agents should be added that lessen the side effects of the combination; and (*e*) the regimen must not adversely affect the antitumor activity or add to the other toxic effects of the chemotherapeutic agents.

Several open trials have been reported with combination regimens. These regimens have generally added steroids to haloperidol (57) or metoclopramide (32–34), with or without additional drugs. Although these have not been comparison studies, to date, the results have been uniformly superior to earlier trials with the agents used singly. More formal studies will be required, but the preliminary results are encouraging and should provide a background for further improvement and trials. It is not clear whether the encouraging activity is due to blocking of several different neuroreceptors (58) or whether it is simply the result of additive antiemetic activity by a variety of mechanisms.

The regimen currently being investigated at our institution combines metoclopramide (2 mg/kg × three doses, or 3 mg/kg × two doses iv) with dexamethasone (20 mg iv given once, 30 minutes before chemotherapy) and diphenhydramine (50 mg iv at the time of the dexamethasone). The latter agent is given prophylactically to lessen the chance of dystonic reactions. To date, this combination has allowed a shorter course of antiemetic treatment, with fewer side effects and a trend toward improved efficacy (34).

CONCLUSIONS

The progress made in antiemetic therapy has helped outline the approach necessary in the future for continued improvement. Single agents must be examined carefully to determine whether they have useful activity, to outline side effects, and to estabish proper doses and scheduling. Only through attention to proper study methods can these goals be achieved. Once the above information is available, comparison trials and combination regimens can be reasonably designed.

Further trials are needed to determine which of the available agents, and which doses, are best for decreasing the emesis associated with specific agents. To date, metoclopramide, corticosteroids, and butyrophenones appear to be the most active agents. Cannabinoids and phenothiazines are superior to placebo, but further examination of different routes of administration and schedules will be necessary to recommend these agents for the emesis induced by the most difficult chemotherapeutic drugs. Several other classes of agents are now being investigated, including benzodiazepines (lorazepam) and some analgesics (butorphanol). Preclinical and preliminary clinical studies have shown interesting activity, which encouraged further trials.

While improvements have been made in acute chemotherapy-induced emesis, only a few studies have approached the difficult problem of anticipatory emesis. Additionally, as treatment regimens become shorter, the problem of delayed or persistent emesis has been more apparent and studies of this issue are clearly needed.

Medical oncologists need to be familiar with the agents that have demonstrated activity and their proper application. Attention to the recommendations of dose and scheduling derived from careful studies is important in achieving maximum efficacy. It is likely that the results of several combination regimens will be appearing in the near future and that treatment options will increase. Evaluating new reports for accuracy of data and applicability to various patient populations will enable the clinician to improve patient care.

REFERENCES

1. CHANG AE, SHILING DJ, STILLMAN RC, ET AL. Delta-9-tetrahydrocannabinol as an antiemetic in patients receiving high-dose methotrexate: a prospective, randomized evaluation. Ann Intern Med 91:819–824, 1979.
2. FRYTAK S, MOERTEL CG, O'FALLON J, ET AL. Delta-9-tetrahydrocannabinol as an antiemetic in patients treated with cancer chemotherapy: a double comparison with prochlorperazine and a placebo. Ann Intern Med 91:825–830, 1979.
3. SALLAN SE, CRONIN CM, ZELEN M, ET AL. Antiemetics in patients receiving chemotherapy for cancer: a randomized comparison of delta-9-tetrahydrocannabinol and prochlorperazine. N Engl J Med 302:135–138, 1980.
4. GRALLA RJ, ITRI LM, PISKO SE, ET AL. Antiemetic efficacy of high-dose metoclopramide: randomized trials with placebo and prochlorperazine in patients with chemotherapy-induced nausea and vomiting. N Engl J Med 305:905–909, 1981.
5. HOMESLEY HD, GAINEY JM, JOBSON VN, ET AL. Double-blind placebo-controlled study of metoclopramide in cisplatin-induced emesis. N Engl J Med 307:250–251, 1982.
6. STRUM SB, MCDERMED JE, OPFELL RW, ET AL. Intravenous metoclopramide. An effective antiemetic in cancer chemotherapy. JAMA 247:2683–2686, 1982.
7. DANIELS M, and BELT RJ. High-dose metoclopramide as an antiemetic for patients receiving chemotherapy with cisplatin. Oncol Nurs Forum 9:20–22, 1982.
8. GOSLIN RH, and GARNICK MB. Metoclopramide as an antiemetic in patients receiving cisplatin. *In* The Treatment of Nausea and Vomiting Induced by Cancer Chemotherapy (Poster D, ed). New York, Masson Publishing, 1981, pp 159–165.
9. LUCAS VS, and LASZLO J. Delta-9-tetrahydrocannabinol for refractory vomiting induced by chemotherapy. JAMA 243:1241–1243, 1980.
10. SALLAN SE, ZINBERG NE, and FREI E. Antiemetic effect of delta-9-tetrahydrocannabinol in patients receiving cancer chemotherapy. N Engl J Med 293:795–797, 1975.
11. GRALLA RJ. Metoclopramide. A review of antiemetic trials. Drugs 25:1:63–73, 1983.
12. GRALLA RJ, BRAUN TJ, SQUILLANTE A, ET AL. Metoclopramide: initial clinical studies of high dosage regimens in cisplatin-induced emesis. *In* The Treatment of Nausea and Vomiting Induced by Cancer Chemotherapy (Poster D, ed). New York, Masson Publishing, 1981, pp 167–176.
13. LEWIN M, MEYER B, DRAYER M, ET AL. Optimizing metoclopramide control of cis-platinum induced emesis. Proc ASCO 1:97, 1983.
14. CAREY MP, BURISH TG, and BRENNER DE. Delta-9-tetrahydrocannabinol in cancer chemotherapy: research problems and issues. Ann Intern Med 99:106–114, 1983.
15. HAYES DM, CVITKOVIC E, GOLBEY RB, ET AL. High-dose cis-plati-

num diammine dichloride: amelioration of renal toxicity by mannitol diuresis. Cancer 39:1372-1381, 1977.

16. LASZLO J. Nausea and vomiting as major complications of cancer chemotherapy. Drugs 25:1-7, 1983.
17. PINDER RM, BROGDEN RN, SAWYER PR, ET AL. Metoclopramide: a review of its pharmacological properties and clinical use. Drugs 12:81-131, 1976.
18. VON HOFF DD, SCHILSKY R, REICHERT CM, ET AL. Toxic effects of *cis*-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 63: 1527-1531, 1979.
19. CHANG EA, SHILING DJ, STILLMAN RC, ET AL. A prospective evaluation of delta-9-tetrahydrocannabinol as an antiemetic in patients receiving adriamycin and cytoxan chemotherapy. Cancer 47:1746-1751, 1981.
20. KRIS MG, TYSON LB, GRALLA RJ, ET AL. Extrapyramidal reactions with high-dose metoclopramide. N Engl J Med 309:433, 1983.
21. TYSON LB, CLARK RA, and GRALLA RJ. High-dose metoclopramide: control of dacarbazine-induced emesis in a preliminary trial. Cancer Treat Rep 66:2108, 1982.
22. PENTA JS, POSTER DS, and BRUNO S. Cancer chemotherapy-induced nausea and vomiting: a review. *In* Treatment of Cancer Chemotherapy Induced Nausea and Vomiting (Poster D, ed). New York, Masson Publishing, 1981, pp 1-32.
23. FETTING JH, GROCHOW LB, FOLSTEIN MF, ET AL. The course of nausea and vomiting after high-dose cyclophosphamide. Cancer Treat Rep 66:1487-1493, 1982.
24. BORISON HL, and MCCARTHY LE. Neuropharmacology of chemotherapy induced emesis. Drugs 25:8-17, 1983.
25. MORROW GR. Prevalence and correlates of anticipatory nausea and vomiting in chemotherapy patients. JNCI 68:585-588, 1982.
26. MORROW GR, and MORRELL C. Behavioral treatment for the anticipatory nausea and vomiting induced by cancer chemotherapy. N Engl J Med 307:1476-1480, 1982.
27. HARRINGTON RA, HAMILTON CW, BRODGEN RN, ET AL. Metoclopramide: an updated review of its pharmacological properties and clinical use. Drugs 25:451-494, 1983.
28. GYLYS JA, DORAN KM, and BUYNISKI JP. Antagonism of cisplatin induced emesis in the dog. Res Commun Chem Pathol Pharmacol 23:61-68, 1979.
29. FLORCZYK AP, SCHURIG JE, and BRADNER WT. Cisplatin-induced emesis in the ferret: a new animal model. Cancer Treat Rep 66:187-189, 1982.
30. GARNICK MB. Oral metoclopramide and cisplatin chemotherapy. Ann Intern Med 99:127, 1983.
31. MCDERMED JE. A randomized, cross-over comparison of the effectiveness of intravenous (IV) metoclopramide and IV dexamethasone as antiemetic prevention in cancer chemotherapy. Proc ASCO 2:89, 1983.
32. LANE R, MCGEE R, and RIVKIN S. Antiemetic combination (BCDVR) for control of platinum induced emesis. Proc ASCO 1:65, 1982.
33. PLEZIA PM, ALBERTS DS, AAPRO MS, ET AL. Immediate termination of intractable cisplatin induced vomiting with an intensive 5-drug antiemetic regimen. Proc ASCO 2:93, 1983.
34. TYSON LB, GRALLA RJ, CLARK RA, ET AL. Combination antiemetic trials with metoclopramide. Proc ASCO 2:91, 1983.
35. VINCENT BJ, MCQUISTION DJ, EINHORN LH, ET AL. Review of cannabinoids and their antiemetic effectiveness. Drugs 25:52-62, 1983.
36. HERMAN TS, EINHORN LH, and JONES SE. Superiority of nabilone over prochlorperazine as an antiemetic in patients receiving cancer chemotherapy. N Engl J Med 300:1295-1297, 1979.
37. STEELE N, GRALLA RJ, and BRAUN DW, JR. Double-blind comparison of the antiemetic effects of nabilone and prochlorperazine on chemotherapy-induced emesis. Cancer Treat Rep 64:219-224, 1980.
38. LASZLO J, LUCAS US, HANSON DC, ET AL. Levonantradol for chemotherapy-induced emesis: phase I-II oral administration. J Clin Pharmacol 21:51S-56S, 1981.
39. LONG A, MIODUSZEUSKI J, and NATALE R. A randomized double-blind crossover comparison of the antiemetic activity of levonantradol and prochlorperazine. Proc ASCO 1:57, 1982.
40. TYSON L, GRALLA RJ, BORDIN LA, ET AL. Levonantradol: dose finding studies and antiemetic trials in patients treated with cisplatin. Proc ASCO 1:56, 1982.
41. HOMESLEY HD, GAINEY J, JOBSON VW, ET AL. Failure of delta-9-tetrahydrocannabinol and prochlorperazine to control chemotherapy induced nausea and vomiting. Proc ASCO 1:67, 1982.
42. ORR LE, MCKERMAN JF, and BLOONE B. Antiemetic effect of tetrahydrocannabinol. Arch Intern Med 140:1431-1433, 1980.
43. WAMPLER G. The pharmacology and clinical effectiveness of phenothiazines and related drugs for managing chemotherapy-induced emesis. Drugs 25:35-51, 1983.
44. FRYTAK S, and MOERTEL CG. Management of nausea and vomiting in the cancer patient. JAMA 245:393-396, 1981.
45. HIGI M, NIEDERLE N, BIERBAUM W, ET AL. Pronounced antiemetic activity of the antipsychotic drug levomepromacine (L) in patients receiving cancer chemotherapy. J Cancer Res Clin Oncol 97:81-86, 1980.
46. CARR B, PULONE B, BERNARD M, ET AL. A double-blind comparison of metoclopramide (MCP) and prochlorperazine (PCP) for *cis*-platinum (DDP) induced emesis. Proc ASCO 1:66, 1982.
47. RICH WM, ABDULHAYGUL G, and DISAIA PJ. Methylprednisone as an antiemetic during cancer chemotherapy—a pilot study. Gynecol Oncol 9:193-198, 1980.
48. AAPRO MS, and ALBERTS DS. High-dose dexamethasone for prevention of cis-platinum induced vomiting. Cancer Chemother Pharmacol 7:11-14, 1981.
49. LEE BJ. Methylprednisolone as an antiemetic. N Engl J Med 3034:486, 1981.
50. CASSILETH PA, LUSK EJ, TORRI S, ET AL. Antiemetic efficacy of dexamethasone therapy in patients receiving cancer chemotherapy. Arch Intern Med 143:1347-1349, 1983.
51. FRUSTACI S, TUMOLO S, TIRELL U, ET AL. High-dose metoclopramide versus dexamethasone in the prevention of cisplatin induced vomiting. Proc ASCO 2:87, 1983.
52. AAPRO MS, PLEZIA PM, ALBERTS DS, ET AL . Double-blind crossover study of the antiemetic efficacy of high-dose dexamethasone vs high-dose metoclopramide. Proc ASCO 2:93, 1983.
53. GROSSMAN B, LESSIN LS, and COHEN P. Droperidol prevents nausea and vomiting from cis-platinum. N Engl J Med 301:47, 1979.
54. NEIDHART J, GAYEN M, and METZ E. Haldol is an effective antiemetic for platinum and mustard induced vomiting when other agents fail. Proc ASCO 21:365, 1980.
55. NEIDHART J, GAGEN M, WILSON H, ET AL. Comparative trial of the antiemetic effects of THC and haloperidol. J Clin Pharmacol 21:38S-42S, 1981.
56. GRUNBERG SM, GALA KV, KAMPENFELD M, ET AL. Comparison of the antiemetic effects of high-dose intravenous metoclopramide and high-dose intravenous haldol in a randomized double-blind crossover study. Proc ASCO 2:85, 1983.
57. MASON BA, DAMBRA J, GROSSMAN B, ET AL. Effective control of cisplatin-induced nausea using high-dose steroids and droperidol. Cancer Treat Rep 66:243-245, 1982.
58. PEROUTKA SK, and SNYDERS SH. Antiemetics: neurotransmitter receptor binding predicts therapeutic actions. Lancet 1:658-659, 1982.

Advances in the Management of Cancer Pain[1]

Richard Payne and Kathleen M. Foley[2,*]

The study of pain in the cancer patient offers a unique opportunity to use clinical observations to advance biologic knowledge. The cancer patient unfortunately represents the experimental model of pain. This group of heroic patients can help teach us the physiologic and psychologic differences between acute and chronic pain, the importance and evolution of psychologic factors, the difference between pain and suffering, the clinical pharmacology of analgesic drugs, and the behavioral mechanisms humans use to suppress pain. They are a rich resource of research potential which should not go untapped. The development of better methods of pain control will benefit all patients with pain. There is a pressing need to develop innovative approaches based on sound, scientific principles, and advances in research technology offer us the opportunity to understand the complex phenomenon of pain. [Cancer Treat Rep 68:173–183, 1984]

Advances in the diagnosis and treatment of cancer, coupled with recent advances in our understanding of the anatomy, physiology, pharmacology, and psychology of pain perception, have led to improved care of the patient with pain of malignant origin. Specialized methods of cancer diagnosis and treatment provide the most direct approach to treating cancer pain by treating the cause of the pain. However, prior to the introduction of successful antitumor therapy, or when treatment of the cause of the pain has failed, or when significant injury to the bone, soft tissue, or nerve has occurred as a result of therapy, appropriate management of pain is imperative and requires a multidimensional approach.

Recent advances in pain research eclipse what can be readily applied at the clinical level but provide the scientific rationale for the use of new and improved methods of treatment, including better and more effective use of standard drug therapy (non-narcotic, narcotic, and adjuvant analgesic drugs), the development of new drugs and use of novel methods and routes of drug administration, the use of selective anesthetic and neurosurgical approaches, and the integration of behavioral approaches to pain control.

Pain represents a significant problem in one-third of cancer patients in active therapy and in 60%–80% of patients with terminal illness (1). A series of specific pain syndromes have been described detailing the different types of cancer pain, both acute and chronic (table 1). Direct tumor involvement is the major cause of pain in approximately 65% of patients, with pain resulting from cancer therapy (surgery, chemotherapy, or radiation therapy) occurring in 25% of patients (2). Ten percent of cancer patients have pain unrelated to the cancer or the cancer therapy. Physicians' awareness of these pain syndromes and the use of a systematic approach to evaluate pain clinically have improved the diagnostic and therapeutic approaches to cancer pain problems (3–6). Recognition of the importance of psychologic factors in both acute and chronic pain states (7), the development of methods to measure pain (8), and the associated psychologic variables represent areas of current active psychologic research which will provide better approaches to clinically assess such patients.

Advances in Pain Research

In the last 15 years, extensive investigations have refined and expanded our knowledge of ascending and descending central nervous system pathways, which process and modulate nociceptive information (9–13). Ad-

---

[1] Supported in part by Public Health Service grant CA-32897 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. R. Payne is a recipient of the Clinical Scholars Research Training Fellowship Award, National Research Service Award (F32 NSO7285).

[2] Departments of Neurology and Pharmacology, Cornell University Medical College, and Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY.

[*] *Reprint requests to:* Kathleen M. Foley, MD, Department of Neurology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

TABLE 1.—Specific pain syndromes in patients with cancer

- Pain syndromes associated with direct tumor infiltration
  - Tumor infiltration of bone
    - Base of skull syndromes
      - Jugular foramen metastases
      - Clivus metastases
      - Sphenoid sinus metastases
    - Vertebral body syndromes
      - C2 metastases
      - C7, T1 metastases
      - L1 metastases
    - Sacral syndrome
  - Tumor infiltration of nerve
    - Peripheral nerve
      - Peripheral neuropathy
    - Plexus
      - Brachial plexopathy
      - Lumbar plexopathy
      - Sacral plexopathy
    - Root
      - Leptomeningeal metastases
    - Spinal cord
      - Epidural spinal cord compression
- Pain syndromes associated with cancer therapy
  - Postsurgery syndromes
    - Postthoracotomy syndrome
    - Postmastectomy syndrome
    - Postradical neck syndrome
    - Phantom limb syndrome
  - Postchemotherapy syndromes
    - Peripheral neuropathy
    - Aseptic necrosis of the femoral head
    - Steroid pseudorheumatism
    - Postherpetic neuralgia
  - Postradiation syndromes
    - Radiation fibrosis of brachial and lumbar plexus
    - Radiation myelopathy
    - Radiation-induced second primary tumors
    - Radiation necrosis of bone
- Pain syndromes not associated with cancer or cancer therapy
  - Cervical and lumbar osteoarthritis
  - Thoracic and abdominal aneurysms
  - Diabetic neuropathy

vances in our understanding of pain modulation which have clinical implications have been in three areas: the role of neurotransmitters, the role of multiple opiate receptors, and the role of selective analgesic sites.

To consider these advances in perspective, a brief consideration of the anatomy of pain pathways is in order. Receptors uniquely responsive to painful stimuli are found in the skin, muscle, and deeper tissues. These nociceptors excite afferent discharges in two types of nerve fibers, thinly myelinated A-delta fibers and unmyelinated C-fibers. These primary sensory afferents have their cell bodies in the dorsal root ganglion and their axons enter the spinal cord via the dorsal root. They ascend or descend for one or two segments in Lissauer's tract and synapse in specific lamina (lamina I and V) in the dorsal horn. The dorsal horn is an important site for modulating sensory input. Several ascending pathways arise from second-order neurons and decussate in the spinal cord central gray to become the neospinothalamic and paleospinothalamic tracts. These tracts project to discrete regions of the thalamus and cortex. The neospinothalamic tract projects to the ventrobasal complex of the thalamus and has its ultimate projection in the Somatosensory Area II of the parietal cortex. The paleospinothalamic tract sends diffuse projections to the reticular formation and intralaminar nuclei of the thalamus, with its final projection in the frontal cortex. The neospinothalamic pathway subserves pain intensity and localization, whereas the phylogenetically older paleospinothalamic pathway subserves the arousal and emotional components of pain. Descending pathways, the most important of which originate from the periaqueductal gray (PAG) nuclei of the midbrain, synapse in the raphe magnus nucleus of the medulla; from this nucleus a medial pathway, the dorsal longitudinal fasciculus, projects to the dorsal horn to modulate pain transmission. This pathway represents an important descending inhibitory pathway. There is a more laterally placed descending pathway from the locus coeruleus to the dorsal horn which also plays a role in modulation at the spinal cord level.

Role of Neurotransmitters

Neurotransmitters play a significant role in this modulatory system. Substance P is the excitatory neurotransmitter for primary nociceptive afferents (14). Serotonin is the major neurotransmitter in the descending medial pathway. Dopamine, norepinephrine, and the endogenous opioid peptides, enkephalin, β'endorphin, and dynorphin, modulate pain at both the spinal and supraspinal levels. Attempts to enhance or mimic the role of these neurotransmitters in pain modulation have led to a series of clinical analgesic studies of amitriptyline, adjuvant analgesic drugs (butyrophenones and phenothiazines), β'endorphin, and the enkephalin analogs. Amitriptyline has analgesic properties independent of its antidepressant effects and appears to work by enhancing serotonin activity centrally by presynaptic inhibition of its re-uptake (15). Haloperidol and methotrimeprazine have analgesic properties in both animals and man which are mediated in part through the enkephalinergic system (16). Metkephamid, an enkephalin analog (17), and human β'endorphin (18) have been studied in man and have been demonstrated to have analgesic properties, but detailed studies are currently lacking to define the role of these drugs in clinical practice.

Role of Opioid Receptors

The discovery of the opioid receptor, a stereospecific binding site on the end of free nerve endings which binds endogenous and exogenous opioids, is the second major advance. These receptors, whose localization strongly correlates with the anatomy of the ascending and descending pain pathways, mediate the multiple pharmacologic effects of the opioid analgesics. Subpopulations of opioid receptors, including high-affinity and low-affinity mu receptors and delta, kappa, sigma, and epsilon receptors, are the subject of intense investigation (19). The identification of such subclasses of receptors mediating different pharmacologic effects and located in specific cerebral and spinal sites offers the possibility of developing new analgesics targeted for specific receptors, eg, the development of a new drug which produces analgesia independent of respiratory depression or sedation.

Role of Selective Analgesic Sites

The PAG region in the midbrain and the dorsal horn in the spinal cord are areas rich in opioid receptors and represent the supraspinal and spinal sites mediating opioid analgesia. Identification of these areas as selective sites of analgesia is the third major advance. The concept of selective analgesic sites results from studies in both animals and man which show that electric stimulation in the PAG area produces total-body analgesia without motor or sensory changes and that administration of opioids directly into the PAG inhibits pain transmission at the spinal cord level by activating the previously described medial descending inhibitory pathway. The use of brainstem stimulation (20,21) and the administration of the opioid analgesics directly into the cerebrospinal fluid bathing these selective sites in animals and man have been introduced as potentially useful approaches to managing pain. Pain transmission at the spinal cord level can be inhibited by the direct application of morphine, and the recent use of spinal opioid analgesia in clinical pain states exploits these findings (22–25,27–29). These advances in our understanding of the neuroanatomy and neuropharmacology of pain have provided a strong theoretic basis to study pain problems. They have also provided a more scientific rationale for some of the standard approaches currently in vogue.

PHARMACOLOGIC APPROACHES

Concurrent with advances in basic pain research have been important developments in the field of the clinical pharmacology of analgesic drugs. Drug therapy using the non-narcotic, narcotic, and adjuvant analgesics (table 2) represents the mainstay of therapy for patients with acute pain and plays a major role in the management of patients with chronic pain and in the terminal patient with pain. There is a tremendous need to develop rational guidelines for the use of analgesics to improve the management of pain in such patients. While analgesic drugs have many attributes which are of particular advantage in treating patients with cancer, they are not without serious shortcomings, including unsatisfactory durations of pain relief, unacceptable side effects at doses which produce little relief in particular patients, and, with the narcotic analgesics, the development of tolerance and physical dependence. Much of the difficulty encountered with the clinical use of drugs arises from individual variation and differences in response of specific patients to the same drug dose. Several reviews of oral and parenteral analgesics in the management of cancer pain (30–32) have suggested guidelines for the use of these drugs, yet there remains a dearth of information on the clinical pharmacology of the narcotic analgesics. In fact, this lack of specific pharmacologic and pharmacokinetic data has

TABLE 2.—Commonly used drugs in cancer pain management

- *Group 1*: non-narcotic analgesics (nonsteroidal anti-inflammatory drugs)
  - Acetylsalicylic acid (aspirin)
  - Acetaminophen (Tylenol)
  - Phenylbutazone (Butazolidin)
  - Indomethacin (Indocin)
  - Sulindac (Clinoril)
  - Ibuprofen (Motrin)
- *Group 2*: narcotic agonist and antagonist drugs
  - Narcotic agonists
  - Morphine and congeners
    - Morphine
    - Heroin
    - Hydromorphone (Dilaudid)
    - Codeine
    - Oxycodone
    - Levorphanol (Levo-Dromoran)
  - Meperidine and congeners
    - Meperidine (Demerol)
  - Methadone and congeners
    - Propoxyphene (Darvon)
    - Methadone (Dolophine)
  - Mixed agonist-antagonist drugs
  - Partial agonists of the morphine type
    - Profadol
    - Propiram
    - Buprenorphine
  - Partial agonists of the nalorphine type
    - Pentazocine (Talwin)
    - Nalbuphine (Nubain)
    - Butorphanol (Stadol)
- *Group 3*: adjuvant analgesic drugs
  - Anticonvulsants
    - Phenytoin (Dilantin)
    - Carbamazepine (Tegretol)
  - Phenothiazines
    - Methotrimeprazine (Levoprome)
    - Haloperidol (Haldol)
  - Tricyclic antidepressants
    - Amitriptyline (Elavil, Endep)
  - Steroids
  - Antihistamines
    - Hydroxyzine (Vistaril)
  - Amphetamines
    - Dextroamphetamine (Dexedrine)
  - L-Tryptophan
  - Levodopa (Larodopa)

led to several controversies in the pharmacologic management of cancer pain (33). The controversy is focused on three main issues which are summarized below.

Choice of a Narcotic Analgesic and Its Method and Route of Administration

Morphine, heroin, hydromorphone, levorphanol, methadone, codeine, and meperidine are the commonly used analgesics to manage patients with cancer pain. Comparative studies of heroin and morphine in cancer patients with pain demonstrate that by using equianalgesic doses, heroin and morphine have comparable analgesic and mood effects (34). The drugs vary in their chemical structure and pharmacokinetic parameters. Both levorphanol and methadone have long plasma half-lives and long-term use is associated with drug accumulation (35). The role of these drugs in managing patients with pain remains unclarified. There are a series of mixed agonist-antagonist drugs that have not been widely used in the cancer population to study their role and usefulness. No controlled studies have addressed the efficacy of oral liquid narcotic solutions compared to other forms of available oral analgesics. Oral administration has been the standard approach, but the use of parenteral (im and iv), epidural, and intrathecal drug administration may offer certain advantages to cancer patients. The pharmacologic basis for the use of one route versus another as it relates to analgesic efficacy and side effects has not been evaluated.

Development of Tolerance

Cancer patients take narcotic analgesics over a long period, but to what degree the development of tolerance and physical dependence occurs in this population of patients has not been fully addressed. Cross-tolerance does not appear to be complete, and the escalation of drug dosage, switching to alternative drugs, and the use of combination drug therapy are empiric approaches in providing adequate treatment. Rapid escalation of pain is associated with rapid escalation of drug intake, suggesting that pain may play a role in the development of tolerance.

Risk of Substance Abuse

A third controversial issue involves the risk to the cancer patient of developing psychologic dependence on drugs and/or substance abuse. It is this profound fear of causing psychologic dependence that plays a major role in reluctance to prescribe narcotic analgesics in medical illness. These controversies point up the fact that there is a strong need to use carefully controlled clinical studies to develop guidelines of care based on facts, not anecdotal observations or survey data. Advances in cancer pain management will come from the application of the advances in clinical pharmacology to study this group of patients. These advances include the development of well-designed, clinical analgesic assay methods, the development of specific and sensitive analytic techniques to measure opioid analgesics in biofluids, the development of mathematic models to relate pharmacologic and pharmacokinetic parameters of analgesic drugs, and the development of new analgesic drugs and the use of novel routes and methods of administration. To date, these advances have not been widely applied to study cancer pain. A brief description of some of these advances and how they have been used to address some of these controversial issues will be considered.

Development of Clinical Analgesic Assay Methodology

The development of clinical analgesic assay methodology has undergone innovations in experimental design pioneered by Houde (8) in studies of analgesics in cancer patients with pain. The basic principles to which these studies adhere include the importance of controls in the clinical measurement of subjective responses, the double-blind technique, the use of a placebo and a standard of reference along with the test drug, randomization of the order of the administration of the study drug, controlled cross-over data, and statistical validation to establish supposed differences of effects between drugs.

Advances in design to increase study reliability and sensitivity include the use of visual analog scales, which are more sensitive to small changes in effect than categoric scales, the use of multidose observational comparative clinical analgesic evaluation, and the use of visual analog scales to study mood. Using these assay techniques, new analgesic agents can be compared to standard drugs such as aspirin and morphine, providing relative potency values that are necessary to place new drugs in the clinical setting. Such specific assay methodology is lacking for other types of pain relief approaches. Using such an approach, comparative studies of heroin and morphine in cancer patients with postoperative and chronic pain demonstrated that heroin in equianalgesic doses to morphine produces comparable analgesia and comparable side effects (34), but the use of heroin was not associated with significantly different mood effects from morphine.

Development of Specific and Sensitive Analytical Techniques

Another important advance is the development of specific and sensitive analytical techniques to measure exogenously administered narcotic analgesics and endogenous opioid peptides. A series of sensitive and specific radioimmunoassays alone or coupled with the use of high-pressure liquid chromatography as well as the use of mass spectrometry and gas chromatography with electron-capture detectors have improved the ability to determine several of the opioid drugs in biologic tissues. The importance of developing such techniques is exemplified by the studies of heroin metabolism in man which were not possible until recently (36). These studies have helped resolve some of the controversies in the use of oral heroin versus oral morphine, demonstrating that following oral administration of heroin only its metabolite, morphine, can be measured in plasma, whereas following continuous iv infusions of heroin, heroin, 6-monoacetylmorphine, and morphine can be detected in plasma. These studies suggest that oral heroin is an inefficient way of delivering morphine to the cancer patient (37). Another example is the study of the metabolite of meperidine, normeperidine, using specific assay techniques, which has helped to explain the CNS hyperirritability reported in cancer patients receiving meperidine for the treatment of postoperative and chronic pain (38).

Development of Mathematic Models

The third major advance is the development of mathematic models to relate pharmacologic effects to pharmacokinetic parameters. Such models have been developed to study numerous other drugs. Such an approach will allow for prediction of drug doses in patients receiving continuous iv infusions and is based on the minimal effective concentration of drugs to produce analgesic effects. Such studies can also provide information on the pharmacokinetic parameters of side effects, but these methodologic approaches are currently in development and have not been widely used (39–41). Once developed, however, they will provide the scientific basis for the rational dosing of patients with analgesic drugs.

Development of New Drugs

The opioid drugs are classified as either agonist or antagonist drugs, depending on their ability to bind to the opiate receptor and produce analgesia. Morphine is the prototype of the narcotic agonists which interact with the receptor to produce analgesia. The narcotic antagonists are drugs which reverse or block the effects of morphine. Within this latter category of drugs are a series of antagonists with analgesic properties. Narcotic antagonists offer the advantage of being narcotic analgesics which produce less respiratory depression than the narcotic agonist drugs, and their use is associated with a slower development of tolerance and physical dependence. Two fundamentally different types of narcotic antagonists with analgesic properties are now recognized. These two drug classes are distinguished as being either morphine-like or nalorphine-like on the basis of pharmacologic effects and the character of their abstinence syndromes (42). Buprenorphine, a partial agonist of the morphine type, is a new drug of particular interest in that it is active in a sublingual preparation which is ten times more potent than morphine and has a long plasma half-life. Although not available in the United States, this drug has been studied in cancer patients and has been shown to be clinically effective (43,44). In contrast to other narcotic antagonist drugs, its use is not associated with significant psychotomimetic effects. Physical dependence on this drug is less than that associated with the narcotic agonist drugs. Drugs in this class may offer special advantages to the management of patients with cancer and pain, and clinical studies are needed.

Novel Methods and Routes of Drug Delivery

Novel Drug Preparations

The reason to develop novel methods and routes of drug delivery is to attempt to maximize pharmacologic effects and to minimize side effects. In analgesic drug therapy these novel methods of drug administration have been developed to reduce undesirable effects associated with standard routes and methods of administration. The new drug delivery systems include the development of drugs which pass through the stomach and esophagus in an inert form and are then activated on entry into the small intestine. Drugs encased in semipermeable membranes with a lazer-drilled delivery orifice to control the drug delivery, the use of cellulose materials mixed with drug which absorb water at predetermined rates, and the use of ion-exchange resins with or without diffusion-limiting membranes are some of the new technologies in drug preparation. At the present time there are a series of nonsteroidal anti-inflammatory drugs which are being developed for delivery in such a way as to minimize the problems of local esophagitis and gastritis, which are common clinical side effects limiting the usefulness of these drugs in certain groups of patients, particularly the cancer patient.

In addition, there are a series of ongoing studies to evaluate a slow-release form of morphine designed to provide a longer time period of absorption with consequent constant blood levels with a long duration, reducing side effects while increasing the therapeutic effect. Preparation of analgesics in a sublingual form has been used sporadically. The great advantage of this particular technique is that it avoids the first-pass effect in the liver, which for morphine and some of the narcotic analgesics can be significant in reducing active drug available for analgesic efficacy. Buprenorphine, a mixed agonist-antagonist drug previously discussed, can be administered by the sublingual route. This route may be particularly advantageous to the cancer patient who is unable to tolerate drugs because of gastrointestinal obstruction or who has limited venous access or reduced muscle mass for parenteral administration.

Infusion Devices

Continuous iv infusions.—Other methods which have been adapted to the needs of the cancer patient include the use of continuous iv infusions, which can be used in ambulatory as well as in hospitalized patients. These infusion devices consist of a mini-syringe designed to automatically deliver minute amounts of drugs at a constant rate. In the management of patients with cancer pain, continuous infusion of narcotic analgesics may provide better pain control in certain circumstances by providing a constant plasma level of the drug with lower daily drug requirements. Side effects, including respiratory depression, sedation, constipation, and hallucinations, have been reported, but when administered carefully, iv opiate infusions have been demonstrated to be useful. The infusion is titrated to the needs of the individual patient with reported dose ranges for morphine in adults from 1 to 750 mg/hour and in children from 0.6 to 80 mg/hour. Morphine, methadone, meperidine, and hydromorphone have been administered by iv infusion (45-47). The primary deficiency of iv infusion therapy is the inability to predict an ideal maintainence infusion rate and to accommodate for differences among patients. Rapid achievement of an optimal steady-state infusion rate is crucial but at times difficult to obtain.

Continuous sc infusions.—The use of continuous sc morphine infusion by a portable infusion pump is based on the same principle as continuous infusions. It has been used to successfully control the pain of terminal malignancy. Both the subcutaneous and intravenous pumps may be modified so that the patient cannot purposely or inadvertently deliver a bolus of drug or alter the infusion rate. The subcutaneous pump connects to a small-gauge needle which the patient can insert into a new subcutaneous site every day or every other day. Limited pharmacokinetic studies suggest that morphine is completely absorbed and reaches a peak plasma level 20-30 minutes after sc or im administration. Continuous sc infusions may be done outside the hospital and do not require the patency of an intravenous line, offering the obvious advantage over iv infusion (48).

Patient-Controlled Analgesia

This approach is a relatively new investigational technique which has been studied in the management of patients with postoperative pain (39,49). Using this method, patients are able to deliver a preset bolus dose of analgesic iv by pushing a button that activates a programmable drug injector connected to the iv cannula. This method has evolved simultaneously with other approaches in parenteral narcotic drug-delivery methods. The advantage of this system is that it eliminates the cycle of conventional regimens and potential overdose of other administration methods. Numerous studies have demonstrated that patients can effectively titrate their analgesic dose. It has been shown to be safe in obstetric and postoperative pain and has been used in a small population of patients with terminal illness. The effectiveness of patient-controlled analgesia compared with conventional analgesic methods has not been studied. Yet, in the management of patients with acute postoperative pain or acute pain, this particular technique has received widespread patient acceptance. Because it has not been used chronically, the usefulness of this particular technique in the long-term management of patients with chronic cancer pain remains in question.

Spinal Opiate Analgesia

Epidural and intrathecal administration of narcotic analgesics has been employed in the treatment of acute postoperative pain following upper and lower abdominal procedures and in the management of patients with cancer pain. The clinical use of these approaches is based on the demonstration of opiate receptors in the dorsal horn of the spinal cord and on the suppression of activity of spinothalamic tract neurons to noxious stimuli by opiates applied to the spinal cord (22). Localized analgesia is produced without motor blockade. Local spinal epidural and intrathecal injection of relatively small doses of opiates achieves analgesic drug levels at spinal receptors. This approach minimizes the distribution of drugs to receptors in the brainstem and cerebral hemispheres, avoiding the known side effects of systemic administration of opiates, specifically nausea, vomiting, sedation, and respiratory depression.

The clinical use of this approach has been studied following administration of single or multiple doses of opiates to patients with acute postoperative pain and by the use of continuous infusions, both epidurally and intrathecally, in patients with cancer pain. Both the clinical studies and the limited pharmacokinetic data available suggest that neither epidural nor intrathecal administration of opiate analgesics works by a spinal mechanism alone (23-28). Both routes of administration result in significant systemic uptake of the opiates and in rostral redistribution of cerebrospinal fluid to supraspinal sites, with high levels of opiates detected in cisternal cerebrospinal fluid within 1 hour of drug administration (29). The rapid development of tolerance reported in cancer patients with progressive disease and pain following continuous epidural infusion and the significant cross-tolerance induced by systemic opiates make assessment of the role of this approach in cancer pain management difficult. At what point should this approach be considered in the management of the cancer patient with pain? What are the risk/benefit ratios to the individual patient?

There is a pressing need to study the mechanisms of spinal and supraspinal analgesia in cancer patients in an attempt to select what is the appropriate opiate analgesic and/or peptide to maximize analgesia and reduce the occurrence of significant side effects. The development of drugs which would be rapidly degraded in the systemic circulations but produce analgesia at the spinal cord level or drugs that bind selectively to receptors in the spinal cord are potential approaches to consider to overcome the present limitations of this technique.

ANESTHETIC AND NEUROSURGICAL APPROACHES

The new developments in drug therapy coupled with the recognition of the complexity of pain modulation that has emerged over the last 15 years have led to the reassessment of the role of anesthetic and neurosurgical approaches to cancer pain. A detailed review of the current use of these techniques is beyond the scope of this paper. In general, each of these modalities requires a specific expertise to be applied successfully and is most useful in treating patients with well-defined localized pain. Although these procedures have been widely used clinically, controlled studies of their effectiveness as compared to other methods of pain control are lacking. The use of neurolytic anesthetic blocks and neuroablative surgical procedures has decreased, in part related to the neuroanatomic and neurophysiologic data that explain their limitations in the clinical setting and in part related to the fact that patients, as they have become more cognizant of their disease and treatment options, are hesitant to undergo such neurodestructive procedures. They consider the pain an important marker of their disease and do not wish to disrupt the neurologic function mediating such pain. Historically, both anesthetic and neurosurgical procedures for cancer pain have been performed late in the course of a patient's illness, often in debilitated patients, and full evaluation of their effectiveness and duration of action has been thwarted by the development of overriding medical problems. However, one important concept that has emerged is the use of anesthetic and neurosurgical procedures combined with antitumor therapy and analgesic therapy, representing the true multidisciplinary approach to pain management in patients with pain and cancer.

Specific advances in the use of these methods consist of either improved use of existing approaches or the development of new approaches. The use of intermittent and continuous epidural local anesthetic infusions in patients with pain localized to the back and lumbosacral plexus and nitrous oxide to treat incident pain in patients with acute cancer-related pain or in the terminally ill represents old approaches now especially adapted to manage cancer pain. New neurosurgical approaches include central brain stimulation, transcutaneous peripheral nerve stimulation, dorsal root entry zone lesions, and chemical hypophysectomy. Each of these advances will be considered briefly.

Anesthetic Approaches

Intermittent and Continuous Epidural Infusion of Local Anesthetics

This approach has been devised to manage the difficult chronic pain associated with metastatic disease involving the sacrum and/or lumbosacral plexus. The method consists of the intermittent and/or continuous infusion of a local anesthetic into a subcutaneous infusion pump or Ommaya reservoir connected to a catheter which has been permanently placed in the epidural space (50). By varying the amount and concentration of the anesthetic, effective pain relief can be achieved without interruption of significant motor or autonomic function. The risk of infection is minimized because local anesthetics are potent antimicrobial agents. The use of continuous low-dose infusions of local anesthetics is associated with minimal systemic side effects. Further studies on the use of this technique in comparison to standard therapies are necessary to define its place in the management of cancer patients. Its major advantages are that the resultant analgesia is not cross-tolerant with the analgesia produced by narcotic analgesics; and temporary use of this technique allows for the reduction in the amount of systemic opiate drugs, therefore partially reversing the tolerance. This may be a useful preliminary approach in patients in whom the use of spinal opiate analgesia is considered, but in whom large doses of systemic opiates have induced tolerance.

Nitrous Oxide

Nitrous oxide has long been recognized for its analgesic properties but has played a minor role in the management of terminally ill cancer patients until recently (51). Nitrous oxide is administered in oxygen through a nonrebreathing face mask, with concentrations varying from 25% to 75%. This approach was found to be especially useful in the management of children with terminal illness who were refractory to standard pain control measures. Its use in four patients in combination with systemic narcotic analgesics was associated with an improvement of symptoms of pain and anxiety and a demonstrable improvement in alertness. Although long-term nitrous oxide use has been associated with the development of pancytopenia, its short-term use is relatively safe. The number of patients that should be considered for use of this particular approach is small, but it offers one more method in the management of a patient with severe pain inadequately controlled by systemic opiate analgesia. Its most useful role is in the patient whose pain is adequately controlled at rest but is markedly exacerbated by sudden or transient movements. Further studies to define its place in the management of patients with pain and cancer are necessary.

Neurostimulatory and Neuroablative Procedures

Neurostimulatory procedures involving the central and peripheral nervous system represent newer advances in the neurosurgical approaches to pain management. The development of these procedures was based on the concept of central selective sites of analgesia and the fact that stimulation in the PAG, as previously discussed, produces total-body analgesia without interruption of motor or sensory function.

Brainstem and Thalamic Stimulation

Brainstem stimulation has been used to treat patients with chronic pain, including a small number of patients with cancer pain. The technique uses a chronic depth electrode system with an inductive receiver attached to an external transmitter that allows chronic stimulation for pain control (20,21).

In the ten cancer patients treated, effective pain control occurred initially, but tolerance developed to the analgesic effects of stimulation, thereby limiting its chronic use. In two of the ten cancer patients, progressive malignant disease with increasing pain intensity could not be adequately treated with chronic stimulation. The advantages of this procedure are that it can be performed under local anesthesia and that it produces total-body analgesia with minimal side effects. The limited number of patients studied prevents analysis of the complications. More recently, data have been obtained from patients with chronic nonmalignant pain treated with chronic brainstem stimulation that suggest that the analgesic effects produced were not significantly different from placebo controls. The underlying mechanism of the analgesic effect of brainstem stimulation was originally thought to be related to the release of an endogenous morphine-like substance, but placebo control studies suggest that the release of this particular substance is related to the stress of the procedure rather than to a correlation with its analgesic efficacy (Gracely R, unpublished observation).

In contrast, thalamic stimulation has been reported to be useful in the management of cancer patients with pain involving the head and neck region. To date, these clinical reports represent anecdotal observations in uncontrolled series, making it difficult to assess the true analgesic efficacy of this particular approach. The role of neurostimulatory procedures in pain management remains controversial and any future studies should involve the use of placebo controls, detailed assessment of the nature of the cancer pain syndrome, and careful assessment of analgesic efficacy.

Transcutaneous Nerve Stimulation

In 1965, Melzack and Wall's "Gate Theory of Pain" called attention to the potential use of electric stimulation for the management of painful states (9). This technique involves the application of both low- and high-intensity stimulation to peripheral nerves to selectively activate the large-diameter fibers and to close the gate to pain perception centrally. Critical data to support the usefulness of this technique in cancer patients are limited to three studies which suggest that approximately 35%-40% of patients with cancer and pain respond with mild to moderate pain relief (7,52). This technique has been reported to be effective in 75%-80% of patients with acute pain and in 25%-40% of patients with chronic nonmalignant pain.

The advantage of this approach is that it is a safe, noninvasive method with minimal risks and side effects. Its disadvantages are that the analgesia, if it occurs, is transient; tolerance develops to its effect; and effective use demands close patient supervision by trained personnel initially. The high cost of individual stimulators and the limited efficacy data in patients with chronic cancer pain, coupled with the lack of any carefully controlled studies, provide little encouragement to the widespread use of this particular technique.

Neuroablative Procedures

The neuroablative procedures which have recently been used in the management of cancer pain include radiofrequency lesions in the dorsal root entry zone and chemical hypophysectomy. However, cordotomy, either open or percutaneous, remains the most useful neurosurgical procedure to control cancer pain (53). The dorsal root entry zone operation is based on the hypothesis that the central or deafferentation pain that occurs in patients from tumor infiltration of the brachial plexus may originate from the deafferented neuron in the zone of injury. The procedure attempts to selectively destroy the first five Rexed layers which give rise to the secondary neurons for the central projecting pathways (54). Although the use of this procedure remains experimental in the management of patients with selective pain problems, this technique exploits the neuroanatomic components of pain and deserves careful investigation before it is either added to or discarded from the armamentarium of pain treatments.

Chemical Hypophysectomy

Pituitary ablation was first introduced as part of the hormonal therapy for the treatment of hormone-dependent tumors, specifically carcinomas of the breast and prostate (55,56). Objective remission or lack of progression occurs in as many as 30%-40% of patients after this procedure. Dramatic pain relief, independent of antitumor effects, has also been described following surgical hypophysectomy (transphenoidal route) and, more recently, chemical hypophysectomy (alcohol injection)

(57-59). Using alcohol injection directly into the sella turcica, Moricca (57) reported dramatic pain relief in 60% of 600 cancer patients with intractable pain and malignant disease. The use of hypophysectomy has a potentially important role in the management of patients with intractable pain, with widespread metastases including neoplasms that are not hormone-dependent (60). More recent studies report pain relief in anywhere from 35% to 74% of patients undergoing the procedure, with the median duration of 6-7 weeks and maximum duration of 20 weeks (58). The mechanism by which analgesia is produced is not clear but may be the tracking of alcohol up the pituitary stalk into the hypothalamus with consequent disruption of hypothalamic-thalamic endorphinergic pain pathways (59). Potential complications include optic and occular motor cranial nerve dysfunction from a misdirected needle or from suprasella injection of alcohol and the occurrence of diabetes insipidus in about 20% of patients. A lack of detailed clinical data, including follow-up data and the endocrine status of such patients, limits critical assessment of these studies. With both transphenoidal hypophysectomy and chemical hypophysectomy more patients receive pain relief than actually achieve tumor regression, suggesting that endocrine alterations following hypophysectomy are not directly responsible for the pain relief. To support this point, endocrine evaluation following transphenoidal hypophysectomy in 15 patients with metastatic breast cancer revealed that in only one of the 15 was there evidence of total pituitary ablation, whereas in all 15 bone pain diminished or disappeared within 24 hours of operation (61). Such data suggest that interruption of hormonal control does not mediate the analgesic effects. Although the underlying mechanisms remain unknown, attempts to relate the analgesic effects of hypophysectomy to an endogenous pain inhibitory system will provide important information on the central mechanisms of analgesia.

Chemical hypophysectomy does not appear to be technically superior to transphenoidal hypophysectomy in relieving pain and the incidence of diabetes insipidus (10%-20%) is the same for both procedures. Some authors criticize the use of hypophysectomy solely as a pain procedure, making its use controversial at best. Additional clinical study should help to clarify this controversy.

Behavioral Approaches to Pain Control

Probably the single most important advance is that these approaches are now being integrated into the care of the patient with cancer pain. Behavioral pain control techniques such as relaxation training, biofeedback, cognitive control, and hypnosis are being employed in patients with pain and cancer (62), but controlled research involving the use of behavioral therapies in pain treatment or the comparative effectiveness of various behavioral pain control techniques is lacking.

Relaxation Training

Relaxation training with or without the assistance of physiologic biofeedback has been useful in reducing reports of noncancer pain and use of pain medications. The mechanism of action includes reduction of muscle and sympathetically mediated vascular and visceral responses. It also provides the patient with the sense of improved self-control and a calming diversion of attention, breaking the associated pain-anxiety tension cycle.

Biofeedback

Biofeedback provides the patient with information about individual physiologic responses contributing to the pain by using biophysical instrumentation. Patients are trained to alter their behavior in such a way as to change a physiologic response. Several physiologic responses can be measured including alpha waves in the electroencephalogram, finger temperature, and electromyogram on the frontalis muscle. The use of these techniques requires special training and whether biofeedback is superior to any of the other behavioral approaches discussed remains unclarified (63). For some patients it is an effective means of controlling pain but controlled studies are lacking.

Cognitive Behavioral Training

Cognitive behavioral training provides the patient with a variety of coping skills which emphasize the diversion of attention from pain stimulation and the employment of cognitions which are likely to facilitate tolerance, perceived self-control, and adaptive functioning despite pain (64). The effect of these procedures on pain reduction and improved adjustment during illness has been demonstrated in recent years.

Hypnosis

The pain-reduction techniques of hypnotic training overlap those of both relaxation and cognitive behavioral training but add a specific hypnotic induction and a cultural mystique which may facilitate motivation and compliance. Hypnotic training has been demonstrated to be effective in controlling pain in cancer. The clinical literature is based predominantly on case reports reporting that 50% of cancer patients can obtain some relief from hypnosis (65-67). Controlled clinical studies are lacking but the anecdotal evidence is sufficient to suggest its usefulness as an adjunctive method of pain control in such patients. The degree to which a patient can be hypnotized is a major factor in the applicability of this method of pain control, although the level of hypnotic trance is not a reliable indicator for successful pain control. The disadvantages of hypnosis are the large amount of time re-

quired of the physician; the necessity of an experienced, well-trained hypnotherapist; and the lack of subjects who are hypnotizable.

REFERENCES

1. FOLEY KM. Pain syndromes in patients with cancer. *In* Advances in Pain Research and Therapy (Bonica JJ, and Ventafridda V, eds). New York, Raven Press, vol II, 1979.
2. KANNER RM, and FOLEY KM. Patterns of narcotic drug use in a cancer pain clinic. *In* Research Development in Drug and Alcohol Use. Ann NY Acad Sci 362:162-162, 1981.
3. FOLEY KM. The management of pain of malignant origin. *In* Current Neurology (Tyler HR, and Dawson DM, eds). Boston, Houghton Mifflin, 1979, vol 2, chap 18, pp 279-302.
4. ———. Clinical assessment of pain. Acta Anesthesiol Scand 74:91-96, 1982.
5. KORI SH, FOLEY KM, and POSNER JB. Brachial plexus lesions in patients with cancer: clinical findings in 100 cases. Neurology 31:45-50, 1981.
6. KANNER RM, MARTINI N, and FOLEY KM. Incidence of pain and other clinical manifestations of superior pulmonary sulcus tumor (Pancoast's tumors). *In* Advances in Pain Research and Therapy (Bonica JJ, and Ventafridda V, eds). New York, Raven Press, 1982, vol 4, pp 27-38.
7. SPIEGEL D, and BLOOM JR. Pain in metastatic breast cancer. Cancer 52:341-345, 1983.
8. HOUDE RW. Methods for measuring clinical pain in humans. Acta Anesthesiol Scand 74:25-29, 1982.
9. MELZACK R, and WALL PD. Pain mechanisms: a new theory. Science 150:971-974, 1965.
10. BASBAUM AI, and FIELDS HL. Endogenous pain control mechanisms: review and hypothesis. Ann Neurol 4:451-462, 1978.
11. SNYDER SH, and CHILDERS SR. Opiate receptors and opioid peptides. Ann Rev Neurosci 2:35-64, 1979.
12. YAKSH TL. Spinal opiate analgesia: characteristics and principles of action. Pain 11:293-346, 1981.
13. IWAMOTO ET, and MARTIN WR. Multiple opioid receptors. Med Res Rev 1:411-440, 1981.
14. NICOLL RA. Substance P as a transmitter candidate. Ann Rev Neurosci 3:227-268, 1980.
15. WATSON CP, EVAN RJ, REED K, ET AL. Amitriptyline vs placebo in post herpetic neuralgia. Neurology 32:671-673, 1982.
16. BEAVER WT, WALLENSTEIN SL, HOUDE RW, ET AL. A comparison of the analgesic effect of methotrimeprazine and morphine in patients with cancer. Clin Pharmacol Ther 7:436-446, 1966.
17. CALIMLIM JF, WARDELL WM, SRIWALANOKUL K, ET AL. Analgesic efficacy of parenteral metkephamid in treatment of postoperative pain. Lancet 1:1374-1375, 1982.
18. FOLEY KM, KOURIDES IA, INTURRISI CE, ET AL. β-Endorphin: analgesic and hormonal effects in humans. Proc Natl Acad Sci USA 76:5377-5381, 1979.
19. PASTERNAK GW. Opiate, enkephalin and endorphin analgesia: relation to a single subpopulation of opiate receptors. Neurology 31:1311-1315, 1981.
20. HOSOBUCHI Y, ADAMS J, and LINCHITZ R. Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. Science 197:183-186, 1977.
21. RICHARDSON DE, and AKIL H. Pain reduction by electrical stimulation in man. J Neurosurg 47:184-194, 1977.
22. YAKSH TL. The principles behind the use of spinal narcotics. *In* Clinics in Anaesthesiology. Philadelphia, W. B. Saunders, 1983, vol 1, pp 219-232.
23. BULLINGHAM RES, MCQUAY HF, and MOORE RA. Extradural and intrathecal narcotics. Recent Adv Anaesthes Analges 10:141-156, 1983.
24. CAMPORESI EM, and REDICK LF. Clinical aspects of spinal narcotics: postoperative managements and obstetrical pain. *In* Clinics in Anaesthesiology. Philadelphia, W. B. Saunders, 1983, vol 1, pp 57-70.
25. HARBAUGH RE, COOMBS DW, SAUNDERS RL, ET AL. Implanted continuous epidural morphine infusion system. J Neurosurg 56:803-806, 1982.
26. POLETTI CE, COHEN AM, TODD DP, ET AL. Cancer pain relieved by long-term epidural morphine with permanent indwelling systems for self administration. J Neurosurg 55:581-584, 1981.
27. ONOFRIO BM, YAKSH TL, and ARNOLD PG. Continuous low-dose intrathecal morphine administration in the treatment of chronic pain of malignant origin. Mayo Clin Proc 56:516-520, 1981.
28. GREENBERG HS, TAREN J, ENSMINGER MD, ET AL. Benefit from and tolerance to continuous intrathecal infusion of morphine for intractable cancer pain. J Neurosurg 57:360-364, 1982.
29. MAX M, FOLEY KM, INTURRISI CEI, ET AL. Epidural and intrathecal opiates: distribution in CSF and plasma and analgesic effects in cancer patients. Ann Neurol, in press.
30. FOLEY KM. The practical use of narcotic analgesics. *In* The Medical Clinics of North America (Reidenberg MM, ed). Philadelphia, W. B. Saunders, 1982, pp 1091-1104.
31. SHIMM DS, LOGUE GL, MALTBIE AA, ET AL. Medical management of chronic cancer pain. JAMA 241:2408-2412, 1979.
32. BEAVER WT. Management of cancer pain with parenteral medication. JAMA 244:2653-2657, 1980.
33. FOLEY KM. Current controversies in the management of cancer pain. *In* NIDA Research Monograph Series 36:169-181, 1981.
34. KAIKO RF, WALLENSTEIN SL, ROGERS A, ET AL. Analgesic and mood effects of heroin and morphine in cancer patients with postoperative pain. N Engl J Med 304:1501-1505, 1981.
35. DIXON R, CREWS T, MOHACSI C, ET AL. Levorphanol: radioimmunoassay and plasma concentration profiles in dog and man. Res Commun Chem Pathol Pharmacol 29:535-548, 1980.
36. UMANS JG, CHIU TSK, LIPMAN RA, ET AL. Determination of heroin and its metabolites by high-performance liquid chromatography. J Chromatogr 233:213-225, 1982.
37. INTURRISI CE, MAX M, FOLEY KM, ET AL. Heroin: disposition in cancer patients. Clin Pharmacol Ther 31:235-241, 1982.
38. KAIKO RF, FOLEY KM, GRABINSKI, ET AL. Central nervous system excitatory effects of meperidine in cancer patients. Ann Neurol 13:180-185, 1983.
39. GRAVES DA, FOSTER TS, BATENHORST RL, ET AL. Patient controlled analgesia. Ann Intern Med 99:360-366, 1983.
40. DAHLSTROM BE, PAALZOW LK, SEGRE G, ET AL. Relation between morphine pharmacokinetics and analgesia. J Pharmacokinet Biopharm 6:41-53, 1978.
41. PAALZOW LK. Pharmacokinetic aspects of optimal pain treatment. Acta Anaesthesiol Scand 74:37-43, 1982.
42. HOUDE RW. Analgesic effectiveness of the narcotic agonist-antagonists. Br J Clin Pharmacol 7:297S-308S, 1979.
43. ROBBIE DS. Buprenorphine in cancer pain. Br J Clin Pharmacol 7:315S-318S, 1979.
44. VENTAFRIDDA V, DECONNO F, GUARISE G, ET AL. Chronic analgesic study on buprenorphine action in cancer pain—comparison with pentazocine. Drug Res 33:587-590, 1983.
45. MISER AW, MISER JS, and CLARK BS. Continuous intravenous infusion of morphine sulfate for control of severe pain in children with terminal malignancy. J Pediatr 96:930-932, 1980.
46. DECHRISTOFORO R, CARDEN BJ, HOOD JC, ET AL. High dose morphine infusion complicated by chlorobutanol-induced somnolence. Ann Intern Med 98:335-336, 1983.
47. FRASER DG. Intravenous morphine infusion for chronic pain. Ann Intern Med 93:781-782, 1980.
48. CAMPBELL CF, MASON JB, and WEILER JM. Continuous subcutaneous infusion of morphine for the pain of terminal malignancy. Ann Intern Med 98:51-52, 1983.
49. JAMESON A, HARTIG P, FAGERLUND G, ET AL. Patient-controlled analgesic therapy: clinical experience. Acta Anaesthesiol Scand 74:157-160, 1982.

50. PILON RN, and BAKER AR. Chronic pain control by means of an epidural catheter. Cancer 37:903-905, 1976.

51. FOSBURG MT, and CRONE RK. Nitrous oxide analgesia for refractory pain in the terminally ill. JAMA 250:511-513, 1983.

52. VENTAFRIDDA V, SGANZERLA EP, FOCHI C, ET AL. Transcutaneous nerve stimulation in cancer pain. *In* Advances in Cancer Pain (Bonica J, ed). New York, Raven Press, 1979, in press.

53. VENTAFRIDDA V, DECONNO F, and FOCHI C. Clinical percutaneous cordotomy. Adv Pain Res Ther 4:185-198, 1982.

54. NASHOLD BS, and OSTDAHL RH. Dorsal root entry zone lesion for pain relief. J Neurosurg 51:59-69, 1979.

55. LUFT R, OLIVECRONA H, IKKOS D, ET AL. Hypophysectomy in the management of metastatic cancer of the breast. *In* Endocrine Aspects of Breast Cancer (Currie A, ed). Edinburgh, E & S Livingstone, 1958, pp 27-35.

56. PEARSON OH, and RAY BS. Hypophysectomy in advanced breast cancer. Am J Surg 99:544-552, 1960.

57. MORICCA G. Chemical hypophysectomy for cancer pain. Adv Neurol 4:707-714, 1974.

58. LLOYD JW, RAWLINSON WAL, and EVANS RJD. Selective hypophysectomy for metastatic pain. Br J Anaesthesiol 53:1129-1133, 1981.

59. MILES J, and LIPTON S. Mode of action by which pituitary alcohol injection relieves pain. Adv Pain Res Ther 1:867-869, 1976.

60. TINDALL GT, NIXON DW, CHRISTY JH, ET AL. Pain relief in metastatic cancer other than breast and prostate gland following transphenoidal hypophysectomy. J Neurosurg 47:659-662, 1977.

61. LAROSSA JT, STRONG S, and MELBY JC. Endocrinologically incomplete transethnoidal transphenoidal hypophysectomy with relief of bone pain in breast cancer. N Engl J Med 298:1332-1335, 1978.

62. CHAPPELL MN, and STEVENSON TI. Group psychological training in some organic conditions. Ment Hygiene 20:588-585, 1936.

63. TURK DC, MEICHENBAUM DH, and BERMAN WH. Application of biofeedback for the regulation of pain: a critical review. Psychol Bull 86:1322-1341, 1979.

64. RYBSTEIN-BLINCHIK E. Effects of different cognitive strategies on chronic pain experience. J Behav Med 2:93-98, 1979.

65. HILGARD ER, and HILGARD JR. Hypnosis in the Relief of Pain. Los Altos, CA, William Kaufmann, Inc, 1975.

66. KOERNER ME. Using hypnosis to relieve pain of terminal cancer. Hypnosis Quart 20:39-46, 1977.

67. BARBER J, and GITELSON J. Cancer pain: psychological management using hypnosis. Cancer 30:130-135, 1980.

Importance of Prognostic Factors in Cancer Clinical Trials

Richard Simon[1,*]

The importance of prognostic factors in the design, analysis, and reporting of clinical trials is discussed. For many kinds of cancer, the variability in prognosis among patients is greater than the size of treatment differences usually seen. Consequently, failure to understand and adequately account for patient heterogeneity easily leads to unreliable claims and inefficient trials. Identified prognostic factors are generally of sufficient importance to demand attention in design and analysis, but rarely are sufficiently explanatory to render randomization unnecessary. Improvement in knowledge of prognostic factors is important for sharpening the focus and for improving the reliability, efficiency, and interpretability of clinical trials. Problems in the conduct of prognostic factor studies are also discussed, and the calculation of the proportion of variability explained by logistic regression models is illustrated for two examples. [Cancer Treat Rep 68:185–192, 1984]

The natural history and response to treatment of many kinds of human cancer are quite variable. Although some of the variability in response is due to differences in treatment administration and methods of assessment, differences in patient and tumor characteristics are also major factors. For many solid tumors, variability in prognosis among patients is more substantial than improvements in prognosis due to therapy. This has major implications for the conduct of clinical cancer research. Failure to adequately appreciate the importance of patient selection factors contributes to the design of inefficient studies, the erroneous interpretation of results, and the development of an inconsistent literature. In this paper, I shall discuss some of the implications of patient heterogeneity and comment on methodology for the development and application of knowledge about prognostic factors.

Variability in Prognosis

Although the public tends to view cancers as uniform in outcome, considerable variability in clinical course and response to therapy often exists. The extent of variability depends on the type of cancer, class of patients, and kind of endpoint. Some diseases like breast cancer are notoriously heterogeneous, although patients with metastatic breast cancer are quite similar with regard to the probability of surviving > 3 years. Some diseases which were at one time considered uniformly and rapidly fatal are now much more variable as a result of advances in treatment.

Variability in prognosis and response is a substantial problem for the treatment of patients and the conduct of clinical research. Thus, the study of sources of variability is important. As sources of variability are identified, this information can be used to improve the design of studies and the reproducibility of results. In this section, a few illustrative examples of variability will be cited. Table 1 shows the tremendous variability in reported response rates to 5-FU administered by rapid injection to patients with advanced cancer of the large bowel (1). Much of this variability is probably due to subjectivity of response assessment and differences in data reporting, but some of it is certainly due to patient selection factors. Reliable evaluation of drug efficacy is practically impossible with this type of uncontrolled variability.

Table 2 shows tumor response rates as a function of initial ambulatory status for seven tumor sites (2). It is well-established that ambulatory status and prior treatment are dominant prognostic indicators of response in the chemotherapy for most types of cancer. Ambulatory status also influences the results of phase II studies in a more subtle way. Very debilitated patients may die before receiving treatment sufficient to evaluate the

---

[1] Biometric Research Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[*] *Reprint requests to:* Richard Simon, PhD, Biometrics Research Branch, Division of Cancer Treatment, National Cancer Institute, Landow Bldg, Rm 4B06, 7910 Woodmont Ave, Bethesda, MD 20814.

TABLE 1.—Results of treatment with 5-FU for patients with advanced carcinoma of the large bowel (ref 1)

Reporting group	No. of patients treated	Objective regression rate (%)
Sharp and Benefiel	13	85
Hall and Good	19	63
Rochlin et al	47	55
Allaire et al	17	47
Cornell et al	13	46
Eyerly	12	42
Field	37	41
Bell	22	36
Weiss and Johnson	37	35
Ferguson and Humphrey	12	33
Hurley	150	31
Eastern Cooperative Oncology Group	48	27
Brennan et al	183	23
Hyman et al	30	20
Ansfield	141	17
Mayo Clinic	144	15
Ellison	87	12
Kennedy	22	9
Knoepp et al	11	9
Olson and Greene	12	8

chemosensitivity of their tumor. The lack of standardization in accounting for such patients when calculating response rates contributes to variability in reported results.

Figure 1 shows marrow remission duration curves for three risk groups of patients with acute lymphocytic leukemia. The risk groups are defined on the basis of age and initial leukocyte count, and results are shown for several treatment protocols (3). Although the Children's Cancer Study Group has since developed a more refined risk-group categorization, it is clear that the major differences in prognosis for the simple grouping in figure 1 are of major importance for the treatment of patients and conduct of clinical research.

Figure 2 shows survival curves reported by Byar (4) for patients with thyroid, prostate, lung, and brain cancers, each stratified by prognostic groups. Whereas the differences in survival among the groups might be somewhat less extreme in subsequent confirmatory studies, it is clear that enormous variability in outcome exists for each type of cancer.

Figure 3 shows a block diagram of a 2-year relapse rate categorized by histology, age, nodal status, and type of chemotherapy for children with Wilms' tumor (5). Again, the influence of patient selection factors is substantial.

Figure 4 shows the effect of age and stage on the probability of surviving 2 years from diagnosis for patients with neuroblastoma. The curves represent smoothed regression representations of the actual data (6).

Design of Clinical Trials

Knowledge of prognostic factors is important for stimulating therapeutic developments and targeting clinical trials to appropriate kinds of patients. Being able to distinguish patients who do well from those who do poorly permits one to focus attention on reducing complications for the former and to develop alternative approaches for the latter. It also permits one to avoid exposing patients with good prognoses to dangerous interventions. In the absence of such predictive ability, the development of improved therapy is constrained by the relative inefficiency of therapeutic evaluations and the fact that improvements in average efficacy may represent retrogressions for subsets of patients. Clinical trials in childhood acute lymphocytic leukemia and the use of hormone-receptor assays in breast cancer are two examples of the use of prognostic factors for improving the focus of therapeutic evaluations.

If eligibility criteria for a protocol include patients who vary substantially in prognosis, it is important to determine beforehand whether one's goals will be limited to determining the relative efficacy of the treatments, on the average, for the mixture of the types of patients included, or whether relative treatment efficacy should be evaluated separately for specified subsets of patients. In the latter case, the sample size for subsets must be sufficient for separate analysis and the subsets should be clearly identified in advance. Sample sizes determined for the former case will generally not be adequate to reliably determine whether relative efficacy varies among subsets. Sometimes subsets of patients are included to

TABLE 2.—Summary of tumor responses as a function of initial ambulatory status for 7 tumor sites (ref 2)

Tumor site	Study No.	No. of responses/No. of patients treated	
		Ambulatory	Nonambulatory
Lymphosarcoma	1472	104/159 (0.65)	15/33 (0.45)
Hodgkin's disease (stages III and IV)	2472	53/64 (0.83)	16/24 (0.67)
Histiocytic lymphoma	3472	20/43 (0.47)	7/19 (0.37)
Sarcoma	1372	10/48 (0.21)	7/58 (0.12)
Malignant melanoma (internal disease)	1071, 1672	20/113 (0.18)	5/43 (0.12)
Colon-rectum	0870	11/94 (0.12)	3/90 (0.03)
Breast	0971	23/54 (0.43)	4/33 (0.12)
Total		241/575 (0.42)	57/30 (0.19)

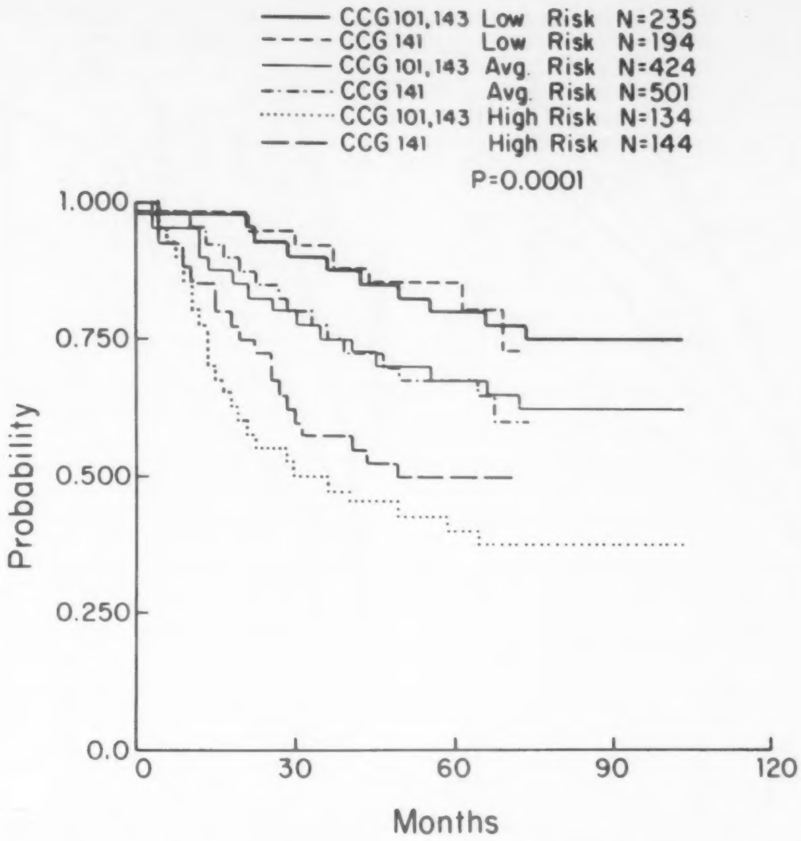


FIGURE 1.—Distribution of bone marrow relapse times by prognostic groups based on age and leukocyte count for children with acute lymphocytic leukemia in Children's Cancer Group studies 101,143 and 141 (ref 3).

build up numbers, with the proviso that they will be "stratified for." Stratification ensures equal heterogeneity but does not ensure that the conclusion "on the average" is appropriate for the constituent subsets. It should be anticipated that important differences in relative treatment efficacy may exist for subsets that differ substantially in prognosis. Major clinical trials should attempt to establish sample sizes sufficient for separate analysis of such subsets. Even in phase II studies where the comparison is to a target response rate rather than another treatment, if previously untreated patients are included, they should be of sufficient number for meaningful separate analysis. Distinguishable subsets of patients which do not vary substantially in prognosis should generally be included without stratification.

The issue of stratified randomization is somewhat controversial among statisticians. For very large studies, stratified randomization is not necessary, but for most smaller trials it is useful (7). Adaptive stratification methods (8,9) permit more factors to be balanced for than do conventional procedures. Good knowledge of prognostic factors permits one to stratify for the truly major independent prognostic determinants rather than to include multiple factors of potential influence. If a regression model relating baseline characteristics to prognosis is available, the model prediction for individual patients can be used as a single stratification factor (10).

Phase III evaluations of a new therapy require a control group. When there is substantial variability in patient outcome, it is rarely reasonable to assume that the distribution of prognosis is stable over time and distance. Referral patterns, selection factors, and staging procedures differ among institutions and change with time. An essential assumption made in comparing an experimental treatment to a control is that prognostic factors not explicitly matched for (or otherwise controlled) in the an-

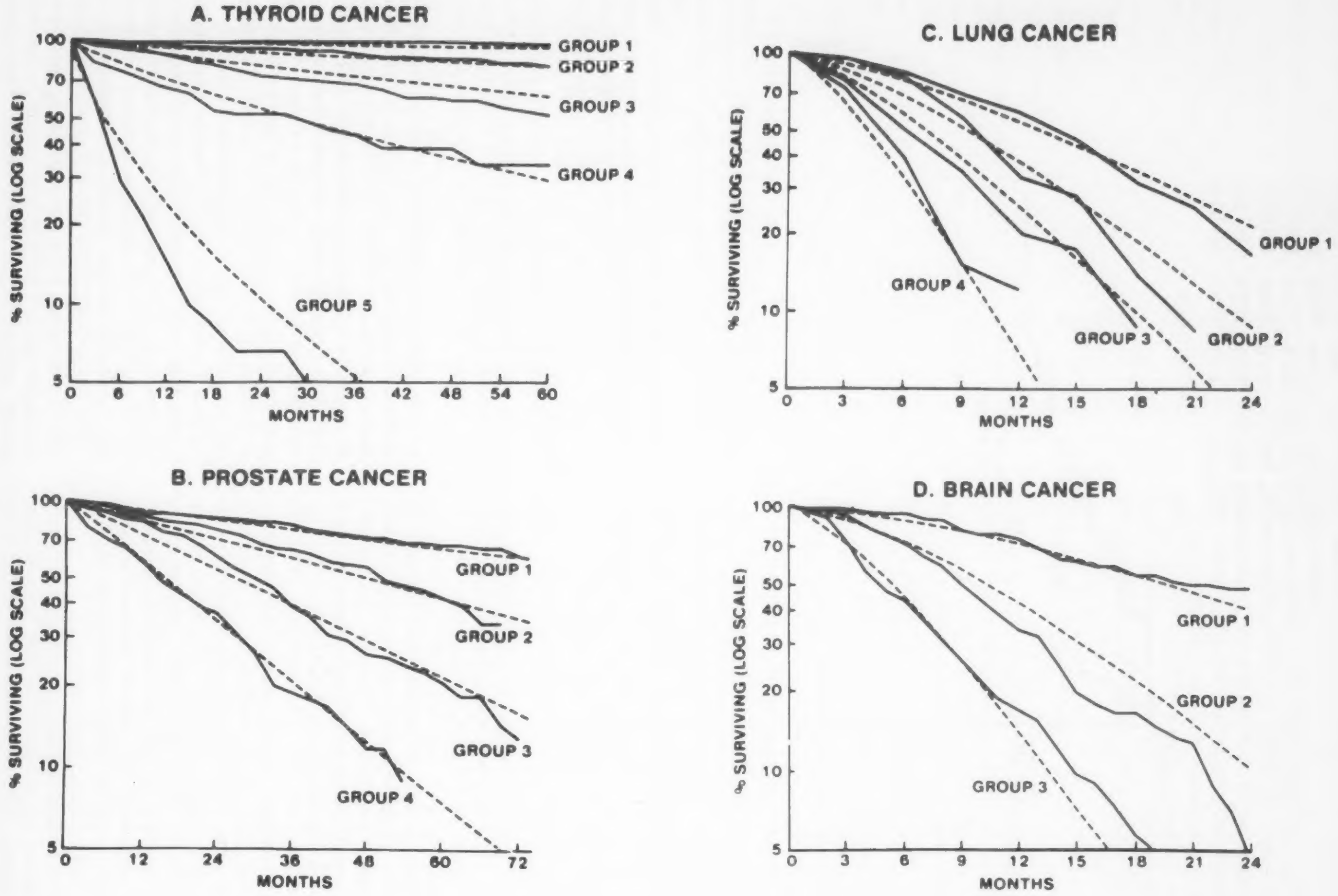


FIGURE 2.—Survival curves by prognostic group for 4 kinds of cancer as reported by Byar (ref 4).

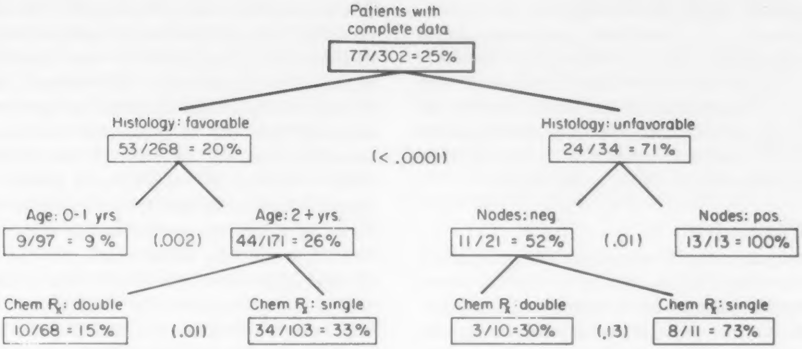

Patients with complete data
77/302 = 25%
Histology: favorable
53/268 = 20%
(< .0001)
Histology: unfavorable
24/34 = 71%
Age: 0-1 yrs
9/97 = 9%
(.002)
Age: 2 + yrs
44/171 = 26%
Nodes: neg
11/21 = 52%
(.01)
Nodes: pos
13/13 = 100%
Chem R_x: double
10/68 = 15%
(.01)
Chem R_x: single
34/103 = 33%
Chem R_x: double
3/10 = 30%
(.13)
Chem R_x: single
8/11 = 73%

FIGURE 3.—2-yr relapse rates for 302 patients with Wilms' tumor. *P* values showing the significance of the difference at each split are in parentheses (ref 5).

alysis can be regarded as having the same distribution for each treatment group. The validity of this assumption is assured by randomization. Without randomization it is only an assumption and one must try to utilize a control group obtained under conditions favorable to its validity.

Recognizing the importance of variability in outcome is crucial in the proper planning of nonrandomized studies. A control group of patients is required for whom detailed individual data are available for making a critical evaluation of eligibility and comparability. Ideally, the determi-

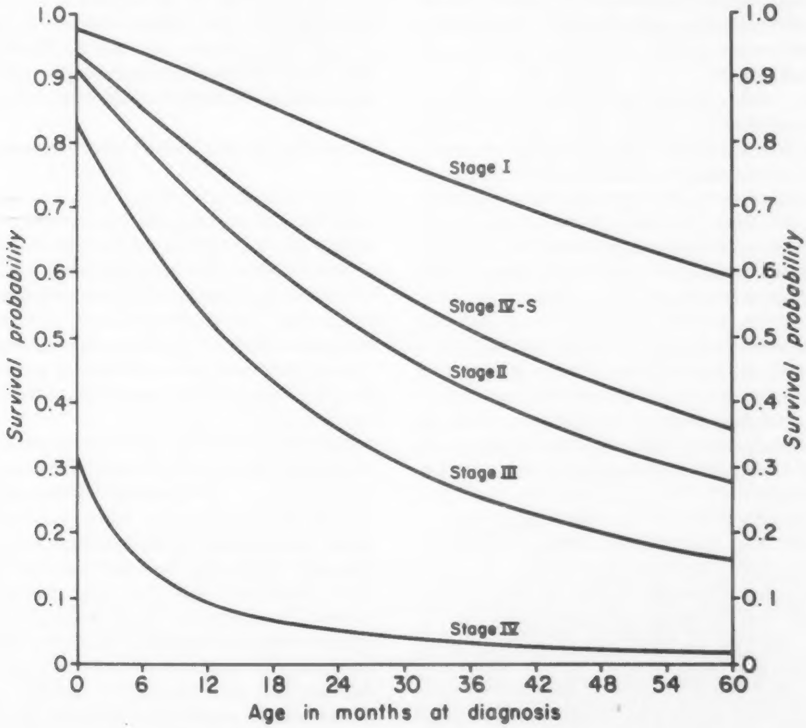


FIGURE 4.—Probability of surviving 2 yrs from diagnosis of neuroblastoma as a function of age and stage. From regression analysis performed by Breslow and McCann (ref 6).

nants of prognosis would be so completely understood that the opportunity for prognostic noncomparability would be minimal. This is rarely the case. The process of properly assembling and evaluating a nonrandomized control group is a substantial effort, which requires sophistication for identifying sources of bias as well as ability to separate the desire to proceed with a study from the critical assessment of adequacy of the design.

Analysis of Clinical Trials

When there is substantial variability in outcome among patients, treatment comparisons must account for important prognostic factors. This is particularly critical for nonrandomized trials, for there is no a priori reason to expect comparability in prognosis. For many such comparisons reported in the literature, there is gross inadequacy in the documentation of prognostic comparability. For such studies, the magnitude of treatment differences that do exist may be minor compared to prognostic differences which can distort the comparisons. Some of the confusion in the literature that results from poorly performed nonrandomized studies might be alleviated if journals required that: (*a*) nonrandomized studies used as a basis for comparative conclusions be reported accompanied by specific data for a well-described control series; and (*b*) description of outcome be provided in a more detailed stratified manner.

Randomized trials are also unreliable when there is substantial variability in outcome, if they are not of sufficient size and do not deal appropriately with major prognostic factors. Displaying comparability of the treatment groups with regard to major prognostic factors is necessary but not sufficient. Even though there may be good comparability, the variability in outcome due to patient factors may mask the difference due to treatment (7). It is fairly common to ignore prognostic factors after demonstrating that the two treatment groups are not "significantly different" with regard to the factors. This is not appropriate (11). Statistical significance is a measure of the likelihood that a given difference could have arisen by chance; it does not measure the extent to which the difference in prognostic factors could be distorting the comparison of treatments with regard to outcome. A statistically nonsignificant difference for a very important prognostic variable can have a very distorting effect.

There are two basic approaches to incorporating major prognostic factors into the analysis of results. The stratification approach involves partitioning the patients into disjoint subsets determined by major prognostic factors, calculating a measure of treatment difference within each subset, and averaging these differences across subsets. In this way a single measure of average treatment difference is obtained which is not diluted by the variability due to prognostic factors. The use of the Mantel-Haenszel test for comparing survival or response rates in this way is described by Simon (12). The second basic approach to incorporating major prognostic factors into the comparison of treatments is via statistical regression models. The logistic model for response and the Weibull or proportional hazards model for survival are popular methods. The use of regression models in clinical research is discussed by Byar (4) and Simon (13). When there is substantial variability in patient outcome, randomized trials with insufficient numbers of patients may result in erroneous conclusions of no treatment difference. This tendency is exacerbated by the overuse of statistical significance tests rather than by confidence intervals in interpreting results. Confidence intervals can be very easy to calculate (14), and they make it clear when the data are indeterminate. With substantial variability in outcome, it is important to plan sample sizes carefully and to incorporate major prognostic factors into the analysis.

A major problem in the assessment of clinical trial results is the interpretation of subset analyses. Results for major subsets should be reported but interpreted very cautiously unless the trial was designed with sufficient sample sizes for separate analyses. Unless the latter is the case, statistical significance tests should not be performed separately by subset unless an "interaction test" demonstrates that relative treatment efficacy varies among subsets more than can be attributed to chance (15). "Data dredging" by examining numerous subsets is a major source of unconfirmable claims today.

How Well Do We Understand Prognosis?

The literature of prognostic factor studies has three main limitations: (*a*) the large number of studies in which only a single factor or very few factors are examined; such studies may provide key biologic information, but they do not permit the determination of whether the new indicators are closely correlated with other prognostic factors; (*b*) the conduct of prognostic factor studies with inadequate numbers of patients; and (*c*) the statistical methodology used for multivariate prognostic factor studies.

Multivariate studies often overemphasize the statistical significance of results and fail to adequately evaluate the importance or magnitude of effects represented by the factors. Some studies seem to assume that finding many "statistically significant" factors means that a considerable amount has been learned about prognosis. This is not necessarily true. The proportion of variability explained by a predictive model should be quantitated. For logistic regression models with binary data this can be done using the negative of the logarithm of the likelihood function (16) as a measure of residual variability. The proportion of variability explained by a model is $1 - V_1/V_0$, where V_1 is the residual variability for the model and V_0 is the total variability when no explanatory variables are considered. One can have prognostic indicators that are highly statistically significant, but which explain very little of the variability in outcomes. Because the proportion of variability explained by a nonlinear model is rarely reported for prognostic factor studies, the calculation will be illustrated here for two examples.

Table 3 summarizes some results of Smith et al (17) on predicting remission for adults with acute leukemia. Based on a logistic model incorporating the factors age, antecedent hematologic disorder, temperature, BUN, hemoglobin, and liver enlargement, five risk groups are shown for evaluating the fit of the model to the data. We have used these five groups as a prognostic classification and have calculated the average probability of success within each group (p_i). The overall response rate is 181/300 = 0.603. The total variability in the data is measured as

$$-\log(0.603)^{181}(0.397)^{119} = 201.5.$$

The variability not explained by the regression is measured as

$$-\sum \log[p_i^{r_i}(1-p_i)^{n_i-r_i}] = 161.0,$$

where n_i is the number of patients in the i'th group, r_i is the number of responders in the i'th group, and p_i is the average response rate for the i'th group. For these data, the proportion of variability accounted for by the regression is

$$1 - 161.0/201.5 = 20.1\%.$$

The report by George and Hoogstraten (18) evaluated 25 factors for predicting initial response to chemotherapy for patients with advanced breast cancer. A logistic prediction model was developed using the variables of disease-free interval, liver involvement, and performance status, because other statistically significant variables added very little to prediction ability. Although the authors did not report the proportion of variability explained by the model, they did report the log likelihood for the final model (-140.125) and for the model without explanatory variables (-160.223). Thus, the proportion of variability explained by the model is

$$1 - 140.125/160.223 = 12.5\%.$$

The fact that "the important prognostic factors are known" is sometimes used as a justification for performing nonrandomized comparative studies. Known prognostic factors are important enough to demand attention in design and analysis but are rarely sufficiently explanatory to ensure that randomization is unnecessary. It is important to develop better predictors of outcome.

TABLE 3.—Prognostic groups for adults with acute leukemia (ref 16)

Prognosis group	Average response rate: p_i	No. of patients: n_i	No. of responders: r_i
1	0.12	34	7
2	0.30	34	9
3	0.50	57	24
4	0.70	81	59
5	0.87	94	82
		300	181

Reliability of Prognostic Factor Studies

Because prognostic factor studies often examine numerous variables, there can be a serious problem of reliability and reproducibility of results. Using computer simulation, Freedman (19) demonstrated surprisingly high probabilities of finding spurious prognostic associations when the number of examined factors is too large relative to the number of patients. The cancer literature contains many instances of inconsistent results for prognostic factor studies. Some investigators do not recognize that even though a set of data can be fit increasingly well with the inclusion of more and more factors, the ability of the model to accurately predict outcome for future patients decreases as the model becomes overly complex. The addition of more and more statistically significant factors to a regression model may reduce its accuracy for prediction. Approaches to determining how many variables should be included in a regression model are discussed by Simon (13). Prognostic models should be confirmed on an independent set of data when possible. In other cases, cross-validation methodology (13) should be employed to avoid developing overly elaborate models. The usual approach to showing good agreement between observed and fitted results on a single set of data indicates only that the family of models used was rich enough to fit the data; it does not demonstrate the reliability of the predictions. Evaluating adequacy of fit is important, but not in itself sufficient if many factors have been considered relative to the sample size. Developing a reliable understanding of how a disease behaves with regard to prognostic determinants is a very complex and important undertaking, which generally cannot be achieved without detailed analysis on a sizable set of data.

CONCLUSION

One important lesson to be learned from the vast literature of cancer therapy is that patient selection factors can have a greater influence on outcome than the treatments under study. Zelen (2) identifies two groups of investigators who ignore this lesson in the design and analysis of studies. One group is interested only in dramatic effects that overwhelm the influence of all prognostic factors. This approach has had successes of major importance but has also led to confusion and inconsistency. The other group has relied entirely on the fact that randomization produces equal heterogeneity on the average, but it has not focused on improving the precision of comparisons by accounting for the influence of prognostic factors. By a more adequate appreciation of the importance of prognostic factors, both the "home-run" approach and the approach based on stepwise improvements can be made more reliable and efficient. Improving the reporting of results, improving the conduct of prognostic factor studies, and improving the predictive value of prognostic factor models are important components for achieving this increase in reliability and efficiency.

REFERENCES

1. MOERTEL CG, and REITEMEIER RJ. Advanced Gastrointestinal Cancer/Clinical Management and Chemotherapy. New York, Harper and Row, 1969.
2. ZELEN M. Importance of prognostic factors in planning therapeutic trials. *In* Cancer Therapy: Prognostic Factors and Criteria of Response (Staquet MJ, ed). New York, Raven Press, 1975, pp 1-6.
3. MILLER DR, SANFORD L, ALBO V, ET AL. Prognostic factors and therapy in acute lymphoblastic leukemia of childhood: CCG-141. Cancer 51:1041-1049, 1983.
4. BYAR DP. Analysis of survival data: Cox and Weibull models with covariates. *In* Statistics in Medical Research (Mike V, and Stanley KE, eds). New York, Wiley, 1982, pp 365-401.
5. BRESLOW NE, PALMER NF, HILL LR, ET AL. Wilms' tumor: prognostic factors for patients without metastases at diagnosis. Cancer 41:1577-1589, 1978.
6. BRESLOW NE, and MCCANN B. Statistical estimation of prognosis for children with neuroblastoma. Cancer Res 31:2098-2103, 1971.
7. SIMON R. Patient heterogeneity in clinical trials. Cancer Treat Rep 64:405-410, 1980.
8. POCOCK SJ, and SIMON R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 31:103-115, 1975.
9. BEGG CB, and IGLEWICZ B. A treatment allocation procedure for sequential clinical trials. Biometrics 36:81-90, 1980.
10. MIETTINEN OS. Stratification by a multivariate confounder score. Am J Epidemiol 104:609-620, 1976.
11. ROTHMAN KJ. Epidemiologic methods in clinical trials. Cancer 39:1771-1775, 1977.
12. SIMON R. The design and conduct of clinical trials in oncology. *In* Principles and Practice of Oncology (DeVita VT, Hellman S, and Rosenberg SA, eds). Philadelphia, Lippincott, 1982, pp 198-225.
13. ______. Use of regression models: statistical aspects. *In* Cancer Clinical Trials: Design, Practice and Analysis (Buyse ME, Staquet MJ, and Sylvester RJ, eds). Oxford, Oxford Press, 1983, pp 445-466.
14. SIMON R, and LEE YJ. Nonparametric confidence limits for survival probabilities and median survival time. Cancer Treat Rep 66:37-42, 1982.
15. SIMON R. Patient subsets and variation in therapeutic efficacy. Br J Clin Pharmacol 14:473-482, 1982.
16. EFRON B. Regression and ANOVA with zero-one data: measures of residual variation. J Am Stat Assoc 73:113-121, 1978.
17. SMITH TL, GEHAN EA, KEATING MJ, ET AL. Prediction of remission in adult acute leukemia. Development and testing of predictive models. Cancer 50:466-472, 1982.
18. GEORGE SL, and HOOGSTRATEN B. Prognostic factors in the initial response to therapy by patients with advanced breast cancer. JNCI 60:731-736, 1978.
19. FREEDMAN DA. A note on screening regression equations. Am Stat 37:152-155, 1983.

Recent Concepts of Cancer Metastasis and Their Implications for Therapy

Isaiah J. Fidler[1,*]

Since the initial publication of *Cancer Treatment Reports* a quarter of a century ago, we have witnessed remarkable advances in surgical treatment of primary neoplasms and aggressive adjuvant therapies. Although these developments have increased the success rate for treatment of primary neoplasms, the lethality of most cancers can still be attributed to the propensity of cells from malignant neoplasms to disseminate from their primary site to distant organs and develop into metastases.

There are several reasons for the lack of success in eradication of metastases. First, by the time of diagnosis and initial treatment in the majority of patients with solid malignant tumors, excluding those with some skin cancers, metastasis has probably occurred (1). Second, many metastases are located in organs which are difficult to saturate with therapeutic agents sufficiently selective to destroy tumor cells and not also destroy normal host cells. The third and most formidable obstacle to therapy of metastases may well be the fact that cells populating a primary neoplasm, as well as those in various metastases, are not uniform, but are rather biologically diverse, ie, heterogeneous. This continuous evolution of biologic diversity in neoplasms, which allows variants to be selected, means that primary tumors and metastases can exhibit intralesional and interlesional heterogeneous responses to treatment. The biologic diversity can be generated rapidly in tumors that are either of unicellular or multicellular origin. The evolvement of highly metastatic cells is sufficiently rapid to provide a mechanism for modulation escape from selection pressures, such as therapeutic agents.

The implications of tumor cell diversity for the outcome of treatment of cancer metastasis cannot be overemphasized. For example, by the time of clinical diagnosis, many metastases are fairly large. A lesion at the lower limit of detection by radiography could well measure 1 cm^3, and thus contain in excess of one billion cells. Even the remarkable 99.9% destruction of this lesion would leave one million cells to proliferate and provide a base population for generating new and diverse variants which are likely to be resistant to the modality used to bring about the demise of the majority of the original tumor cells (2).

Collectively, then, it can be stated that the major cause of death from cancer is due not to the primary neoplasm, but rather to metastases which are resistant to conventional therapeutics. Additional and/or new approaches to the therapy of disseminated cancer must be based on understanding the underlying mechanism(s) responsible for the generation of biologic diversity in neoplasms and for cancer spread. Simply stated, logical solutions may be found once a specific problem has been clearly defined.

Biologic Heterogeneity of Malignant Neoplasms

By the time of diagnosis, most animal and human neoplasms consist of subpopulations of cells with widely differing phenotypic characteristics (3). Pathologists have long recognized that primary neoplasms are made up of zones of morphologically distinct cells. For this reason, morphologic characterization of tumors as benign or malignant can be definitively determined only when multiple sections obtained from different areas are examined (4). Recent studies have demonstrated that these zonal differences are not restricted to morphology alone, but include many other biologic characteristics such as antigenic properties (5), sensitivity of cytotoxic drugs (6), and melanogenesis and metastatic properties (7).

There is now overwhelming evidence that clinically apparent malignant neoplasms are heterogeneous (2,3,8-12). Cells obtained from individual tumors have been shown to differ with respect to all biologic characteristics important to the outcome of therapy, such as antigenicity or immunogenicity (13), growth rate (14), cell surface receptors (15), enzyme markers (16), hormone receptors (17), and sensitivity to cytotoxic drugs (6, 18-20). Whether this heterogeneity of cells exists is not controversial; what has been controversial is whether neoplasms are also heterogeneous for metastatic properties. The first direct evidence that this is indeed the case came from the studies of Fidler and Kripke (21) in 1977, which applied the classical fluctuation analysis of Luria

---

[1] Department of Cell Biology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[*] *Reprint requests to:* Isaiah J. Fidler, DVM, PhD, Department of Cell Biology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, HMB 173, Houston, TX 77030.

and Delbruck (22) to the B16 melanoma. We reasoned that if tumors were populated by cells with similar metastatic capacities, then isolated clones would produce equal numbers of metastatic foci; however, if tumors were populated by cells with different metastatic potential, then different clones would produce different numbers of metastases. After cells obtained from mass culture and from isolated clones established from individual cells were injected into syngeneic C57BL/6N mice, the cloned sublines were found to differ markedly from the parent tumor and among themselves in the number of metastases produced. Control subcloning experiments proved that this diversity was not a result of the cloning procedure per se and established that populations of cells with different metastatic capacities existed within the original tumor (21). These results have been confirmed in another murine melanoma of more recent origin (14). Additional evidence that secondary tumor foci develop from subpopulations of cells with heritable metastasis-related characteristics was obtained from studies in which cells that had recovered from metastatic deposits were shown to be more metastatic than their respective heterogeneous parent lines (23). This demonstration of metastatic heterogeneity has since been confirmed in many laboratories, using a wide range of tumors of various histologic types (8,23-25), and implies that only certain subpopulations of tumor cells are capable of completing the arduous and highly selective process of metastasis.

Process of Metastasis

The pathogenesis of cancer metastasis has been the recent subject of several extensive reviews (8,24-26) and will therefore not be discussed in great detail here. To produce a clinically obvious metastasis, tumor cells must survive a number of potentially lethal steps and confrontations with host homeostatic mechanisms, which include defense. Metastasis begins with the local invasion of host stroma by cells from the primary neoplasm. The generation of mechanical pressure, release of degradative enzymes, and increased motility are all part of the mechanisms of invasion. Once the tumor cells penetrate a blood and/or lymph vessel, they can either grow at this site or detach and circulate as small cell clumps or single-cell emboli. While in the circulation, the tumor cells must survive mechanical trauma such as blood turbulence, and evade host immune and nonimmune defenses such as lymphocytes, antibodies, natural killer cells, and free and fixed phagocytes. Those cells which can survive in the circulation must then arrest in the capillary beds of distant organs by adhering either to endothelial cells or to exposed basement membranes (8). Subsequently, tumor cells must extravasate into the organ parenchyma and proliferate to establish new tumor foci. Newly established metastases may give rise to other metastases, the so-called "metastasis of a metastasis" (27), and, in this manner, a small primary tumor can produce a large number of potentially lethal metastases.

The failure of a tumor cell to complete any one of the steps of the metastatic process prevents it from going on to produce a metastasis. This demand for surviving so many difficult interactions may explain, in part, why the process of metastasis has been deemed to be inefficient insofar as tumor cells are concerned (28). For example, using radiolabeled tumor cells, we have observed that by 24 hours after entry into the circulation, < 1% of the cells are still viable, and < 0.1% of tumor cells placed into the circulation survived to produce metastases (29). Observations such as this have prompted us, and many others, to question whether the process of metastasis is random or whether it represents the selective emergence of pre-existent subpopulations of cells endowed with special properties which allow them to survive. In other words, can all cells in a malignant tumor form metastases, or can only specific subpopulations of tumor cells produce secondary lesions? Our studies (21,23) and the majority of studies from other laboratories (3) support the concept that metastasis is a highly selective process that is regulated by a number of imperfectly understood mechanisms. This viewpoint is more optimistic than the one which claims that cancer metastasis is a random process. A random process cannot be manipulated therapeutically. A selective process must be governed by mechanisms that can be studied, dissected, and hopefully understood. As stated above, understanding the biologic basis of metastasis can well lead to improvements in devising therapeutic approaches to this disease.

Origin of Biologic Diversity in Neoplasms

The questions of whether tumors contain cells with different biologic properties and whether metastasis is a nonrandom process no longer merit serious debate (30). The more important issue is to understand the origin of cellular heterogeneity in malignant neoplasms. Tumors could have a multicellular origin and, in this case, their diverse cellular populations would merely reflect diverse parentages (31,32). However, most human cancers probably result from the proliferation of a single transformed cell (33). The generation of biologic diversity in such tumors is probably a consequence of the process of tumor progression, ie, the continuous evolution of tumors.

Clinical and histologic observations of neoplasms have suggested that tumors undergo a series of changes during the course of the disease. It is not uncommon for a tumor that was initially diagnosed as benign over a period of many months or even years to evolve into a malignant tumor. To explain this process of tumor progression (34), Nowell (33) suggested that acquired genetic variability within developing clones of tumors, coupled with host selection pressures, can bring about the emergence of new sublines with increased growth autonomy or malignancy.

This hypothesis predicts that increasing progression towards malignancy will be accompanied by increasing genetic instability of the evolving cells. To test this hypothesis, we have examined the metastatic stability and the rates of mutation to ouabain resistance and/or 6-thioguanine resistance of paired metastatic and nonmetastatic cloned lines isolated from different mouse neoplasms (35). In four diverse tumor systems that we examined, highly metastatic cells were less phenotypically stable than their nonmetastatic counterparts, which were isolated from the same single neoplasm. This rapid generation of diversity may have been caused in part by increased genetic instability. We found that in the highly metastatic clones, the rate of spontaneous mutation to ouabain and 6-thioguanine resistance was severalfold higher than in the poorly metastatic clones (35). These results are in accord with the hypothesis that tumor progression can occur as a result of acquired genetic alterations (33), and similar data have now been published by other investigators (36,37). Further evidence that genetic mechanisms can be responsible for tumor progression comes from recent mutagenesis experiments using nitrosguanidine (38) or ultraviolet radiation (39). Such treatments of tumors have resulted in the emergence of new variants, some of which exhibit increased tumorigenicity and metastatic potential, while others have a decreased tumorigenic potential (38). In any event, our studies show that the more metastatic a population is, the greater the likelihood that those cells will undergo spontaneous mutations, a state of affairs which could well allow for rapid evolution, modulation, and escape from various therapeutic modalities.

Origin of Biologic Diversity in Metastases

Multiple metastases proliferating within the same host can exhibit heterogeneity with regard to many characteristics such as metastatic capacity, hormone receptors, marker enzymes, growth rate, antigenicity, and response to various chemotherapeutic agents (3). Biologic diversity demonstrable among different metastases may be the consequence of the process of tumor evolution or it may result from the nature of tumor cell spread.

As already stated, in general, metastases do not result from the random survival of cells released from the primary tumor; rather, they result from the selective survival and growth of specialized malignant cells that pre-exist in the parent tumor (21). Several questions about the pathogenesis of metastasis remained unanswered. Do tumor emboli that survive the many steps of metastasis to form a distant lesion consist of single cells or cell aggregates? How does the composition of these emboli affect the metastatic process? Can embolic clumps survive in the circulatory system and give rise to homogeneous metastases of unicellular origin?

We performed a series of experiments to determine (*a*) whether individual metastases are clonal in origin and (*b*) whether multiple metastases produced by one tumor can originate from different progenitor cells (40). We examined the metastases that arose from subcutaneously growing x-irradiated cells with induced chromosomal damage. We reasoned that if a metastasis were derived from a single cell, all the spreads examined would exhibit a uniform karyotype. In contrast, if a metastasis were derived from more than one progenitor cell, its constituent cells would exhibit different chromosomal arrangements. A total of 21 metastases were analyzed. In ten lines, all the chromosomes were telocentric and, therefore, these metastases were noninformative. In the other lines, unique patterns of chromosome(s) were found in most spreads, suggesting that each metastasis originated from a single progenitor cell. This experiment did not resolve whether metastases arose as a consequence of individual cells surviving in the bloodstream or whether homogeneous clumps survived in the circulation, but it does indicate that many secondary foci can originate from the progeny of single cells. Moreover, because the metastases each exhibited a unique pattern of marker chromosome, the data also indicate that different metastases could originate from different progenitor cells (40). These data help to explain one source of the biologic diversity of multiple metastases proliferating in the same host.

Our findings that different metastases can originate from different progenitor cells account for the biologic diversity that exists among various metastases. However, even within a solitary metastasis of proven clonal origin, heterogeneity for various characteristics can develop rapidly (41), possibly resulting from the inherent biologic instability associated with clonal populations (41,43) and/or from the high rate of spontaneous mutation associated with cells populating metastases (35).

Phenotypic Instability of Clonal Tumor Populations

Subpopulations of tumor cells within a tumor mass are not autonomous units, but are regulated by the proximity of other neoplastic cells. Different subpopulations of mammary tumor cells have been shown to affect the growth patterns (41) and chemosensitivity of other groups of cells (42,43). Similar regulatory control may exist for the metastatic phenotype of different cells within a heterogeneous tumor (43). To test for this, two clones with low metastatic potential and two with high metastatic potential were isolated from the murine B16 melanoma. These clones were cultured in vitro or in vivo for ten, 20, or 40 passages (5, 10, or 20 weeks), and then subclones derived at each of these intervals were reassessed for their metastatic ability. After only ten in vitro passages, many subclones that differed significantly from the parent clone were isolated. Continued cultivation introduced more variability. In marked contrast, the metastatic phenotype of the uncloned B16 melanoma line remained remarkably stable. Similar stability was attained when different clones were mixed together and co-cultivated as polyclonal populations. However, the destruction of all but one of the clones led to the rapid generation of biologic diversity in that remaining clone (43). These data provide evidence that different subpopulations of tumor cells stabilize their relative proportions and thereby impose an equilibrium on the combined population. Removal of the stabilizing effect by isolating clones or by applying a strong selection pressure, such as chemotherapy, can lead to rapid diversification in the resurgent populations. Although the nature of these stabilizing influences and their mode of action are not yet understood, their very existence argues against randomness in tumor development (30). Collectively, then, the findings that metastatic cells have higher mutation rates than nonmetastatic cells (35) and that heterogeneity develops more rapidly in tumors containing few subpopulations of cells (30,41) suggest that accelerated tumor evolution and progression will result in the rapid development of biologic diversity in solitary metastases, especially when such lesions are of clonal origin (40).

Impact of Heterogeneity on Screening Procedures for New Therapeutic Modalities

The main objective of a screening procedure for anticancer agents is to identify those agents beneficial to cancer patients. Central to the design of a screening procedure is the recognition that the main challenge to the clinical oncologist is the eradication of metastases that occur in the primary host. Two major issues must therefore be considered:

(*a*) *Tumor heterogeneity*.—The existence of specialized subpopulations of cells with high metastatic potential has far-reaching consequences for the testing of potential agents for treatment of metastatic disease. As recently pointed out by Schabel et al (44), many of the methods presently used to define the drug sensitivity of experimental animal tumors might be misleading, since they are based on the belief that transplantable tumors are homogeneous, ie, uniform entities. Partial regression of the "primary tumor" is an inadequate assay for screening agents for their antimetastatic activity. Assays in which a drug or combination of drugs is found to limit growth of a tumor (injected at a "primary site," ie, sc or im) less than in untreated controls merely may reveal the presence of a subpopulation(s) of drug-sensitive cells and does not necessarily offer any insight into the pharmacologic susceptibility of the tumor cell subpopulations that will give rise to metastases (45-47). Efforts to identify potential agents for treatment of metastases might more profitably use modified assays in which therapeutic agents are screened for their activity against tumor cell subpopulations with defined metastatic capabilities.

(*b*) *Host factors*.—Many host factors may influence the proliferation and/or regression of metastases. The role that host defense (local and systemic) plays in the pathogenesis of metastasis is well known and has been the subject of various investigations in our laboratory and many others. In addition, clinical and experimental studies have clearly demonstrated that metastases proliferating in different organs can exhibit different responses to a given regimen of chemotherapy (48,49). Host factors such as neovascularization, availability of growth factors, and inflammatory responses can all influence the interaction of metastatic cells with therapeutic agents.

Implications of Biologic Diversity for Therapy

The two main areas where the biologic heterogeneity of neoplasms is likely to prove of practical importance are in the detection of tumor deposits using cell-produced markers and in therapeutic regimes other than surgical resection and radiotherapy. For example, the presence of growing small cell lung cancer can be detected by elevations in serum levels of calcitonin, histaminase, and L-dopa decarboxylase. When Baylin (16) sampled both primary and secondary tumors for these markers, he found a variable pattern of expression. All primary tumors produced detectable levels of each marker, whereas many of the metastases produced either low or undetectable levels. Findings such as these cast doubt on the feasibility of using any one product of tumor cells in which such diversity in synthesis and/or secretion occurs for the quantitation of disseminated disease.

Equally important, the existence of heterogeneous subpopulations of cells within primary neoplasms presents a dilemma for the therapist. The emergence of drug-resistant variants during or subsequent to chemotherapy has been documented extensively in the clinical literature, and differences in the response of primary and metastatic tumors to therapeutic agents have also been documented (45-47). These differences may be exacerbated by the anatomic location of the secondary tumor deposits, since it is not an infrequent finding that metastases in one organ will be susceptible to chemotherapy, whereas metastases in other organs are resistant (48,49). Similarly, the obstacle to therapy that heterogeneity imposes upon treatment modalities is not restricted to chemotherapy. Even though primary neoplasms may exhibit immunologic heterogeneity (13), there may be additional variations between the primary tumor and its metastases. Such variations can markedly affect the likelihood of success of immunotherapeutic or biologic response modification modalities (50,51).

CONCLUSIONS

The development of metastases, ie, tumor growths at sites or organs distant from the primary cancer, is dependent on an interplay between host factors and intrinsic characteristics of the tumor cells. The pathogenesis of metastasis is a most complex and selective process, and the growth of metastases represents the end point of many destructive events which few tumor cells survive. Only a small number of tumor cells within a primary neoplasm may actually invade blood vessels, and of these, only a few may survive in the hostile circulation. Similarly, not all malignant cells that survive transport can successfully be arrested in the microcirculation, undergo extravasation into the parenchyma, escape host defense mechanisms, and grow into metastases. At every step of the metastatic cascade, the rules of "survival of the fittest" apply with regard to the interplay of tumor cells and host factors.

The most formidable obstacle to the successful treatment of metastases may well be the fact that the cells of metastases are biologically heterogeneous. This phenotypic diversity which allows selected variants to develop means not only that different metastases exhibit different levels of sensitivities to chemotherapeutic drugs (46), but also that even within a solitary metastasis of proven clonal origin, heterogeneity for various characteristics can develop rapidly. This is possible because clonal populations tend to be phenotypically unstable (43) and because cells populating metastases could well have a high rate of spontaneous mutation (35).

A better understanding of the complexity of the processes of tumor evolution and metastasis has not provided immediate solutions to the perplexing problems associated with cancer treatment. However, the following hypotheses are now emerging, and only future data can determine their validity:

(*a*) The heterogeneous nature of a malignant neoplasm has profound implications for tumor therapy that are potentially both significant and disheartening. The heterogeneous response of malignant tumor cell subpopulations, especially to chemotherapeutic drugs, makes it unlikely that a single treatment modality will destroy all the cells in a tumor. Once again, in tumors consisting of 10^9 cells (1 cm^3), the destruction of 99.9% of the population still leaves 10^6 cells to proliferate! From this restricted and perhaps clonal population, variants may develop at an accelerated rate (30) and these newly emerging variants could well be more malignant and more resistant to treatment than the cells in the original tumor (43). This may be more pronounced when cytotoxic drugs which are also mutagenic are used.

(*b*) When dealing with a cancer that, on the basis of accumulated clinical observations, has a high probability of producing metastasis, systemic therapy should commence immediately. Awaiting clinical proof of metastases could well place an overwhelming demand on drugs currently available, since such lesions are fairly large, and, by current analysis, heterogeneous for their chemosensitivity (47) or immunosensitivity (13).

(*c*) The design of therapeutic approaches for metastases should be based on the biologic characteristics of metastases rather than of the primary tumor. Obviously, if few of the cells residing within a tumor are capable of completing the metastatic sequence, then their characteristics may be lost in the general "background level" provided by the nonmetastatic majority. Application of the correct selection procedures or isolation techniques could lead to the emergence of cells in which the measured characteristics are significantly above the background level. Variants of differing biologic activity, established from within the same parent tumor, could then be compared without the need to use so-called normal cells of doubtful validity.

Clearly then, in the last few decades, we have witnessed the development of cytotoxic agents fully capable of destroying the majority of tumor cells in a neoplasm. The lack of selectivity, however, usually prevents such drugs from destroying all tumor cells. The real challenge to cancer researchers is to develop new approaches to the treatment of tumor cells that are resistant to conventional therapies. Such an approach must meet two criteria. First, the treatment must circumvent the problem of cellular diversity. Second, it must obviate the problem of cellular resistance to treatment. We have concentrated our efforts on investigating whether tumoricidal macrophages could fulfill these demanding tasks (50). The validity of this hypothesis is now being actively investigated.

REFERENCES

1. SCHABEL FM. Concepts for systemic treatment of micrometastasis. Cancer 35:15-24, 1975.
2. HART IR, and FIDLER IJ. The implications of tumor heterogeneity for studies on the biology and therapy of cancer metastasis. Biochim Biophys Acta 651:37-50, 1981.
3. FIDLER IJ, and HART IR. Biological diversity in metastatic neoplasms: origins and implications. Science 217:998-1003, 1982.
4. WILLIS RA. The Spread of Tumors in the Human Body. London, Butterworth, 1972.
5. PREHN RT. Analysis of antigenic heterogeneity within individual 3-methylcholanthrene-induced mouse sarcomas. JNCI 45:1039-1045, 1970.
6. TROPE C. Different susceptibilities of tumor cell subpopulations to cytotoxic agents. *In* Design of Models for Testing Cancer Therapeutic Agents (Fidler IJ, and White RJ, eds). New York, Van Nostrand, 1982, pp 64-79.
7. FIDLER IJ, and HART IR. Biological and experimental consequences of the zonal composition of solid tumors. Cancer Res. 41:3266-3267, 1981.
8. NICOLSON GL, and POSTE G. Tumor cell diversity and host responses in cancer metastasis. Curr Probl Cancer 7:4-83, 1982.
9. POSTE G. Experimental systems for analysis of the malignant phenotype. Cancer Metab Rev 1:141-200, 1982.
10. FIDLER IJ, and POSTE G. The heterogeneity of metastatic properties in malignant tumor cells and regulation of the metastatic phenotype. *In* Tumor Cell Heterogeneity (Owens A, et al, eds). New York, Academic Press, 1982, pp 127-145.
11. HEPPNER G. The challenge of tumor heterogeneity. *In* Commentaries on Research in Breast Disease (Bubbrook RD, and Taylor DJ, eds).

New York, Alan R. Liss, 1979, pp 177-191.

12. SPERMULLI EN, and DEXTER DL. Human tumor cell heterogeneity and metastasis. J Clin Oncol 1:496-509, 1983.
13. MILLER FR. Intratumor immunologic heterogeneity. Cancer Metab Rev 1:319-335, 1982.
14. FIDLER IJ, GRUYS E, CIFONE MA, ET AL. Demonstration of multiplephenotypic diversity in a murine melanoma of recent origin. JNCI 67:947-956, 1981.
15. READING CL, BELLONI DN, and NICOLSON GL. Selection and in vivo properties of lectin-attachment variants of malignant murine lymphosarcoma cell lines. JNCI 64:1241-1249, 1980.
16. BAYLIN SB. Clonal selection and heterogeneity of human solid neoplasms. *In* Design of Models for Testing Cancer Therapeutic Agents (Fidler IJ, and White RJ, eds). New York, Van Nostrand Reinhold Co, 1982, pp 50-64.
17. BRENNAN MJ, DONEGAN WL, and APPLEBY DE. The variability of estrogen receptors in metastatic breast cancer. Am J Surg 137:260-262, 1979.
18. CALABRESI P, DEXTER DL, and HEPPNER GH. Clinical and pharmacological implications of cancer cell differentiation and heterogeneity. Biochem Pharmacol 28:1933-1941, 1979.
19. HEPPNER GH, DEXTER DL, DENUCCI T, ET AL. Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mammary tumor. Cancer Res 38:3758-3763, 1978.
20. TROPE C. Different sensitivity to cytostatic drugs of primary tumor and metastasis of the Lewis carcinoma. Neoplasma 22:171-180, 1975.
21. FIDLER IJ, and KRIPKE ML. Metastasis results from preexisting variant cells within a malignant tumor. Science 197:893-895, 1977.
22. LURIA SE, and DELBRUCK M. Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28:491-511, 1943.
23. TALMADGE JE, and FIDLER IJ. Cancer metastasis is selective or random depending on the parent tumor population. Nature 297:593-596, 1982.
24. POSTE G, and FIDLER IJ. The pathogenesis of cancer metastasis. Nature 283:139-146, 1980.
25. FIDLER IJ, GERSTEN DM, and HART IR. The biology of cancer invasion and metastasis. Adv Cancer Res 28:149-250, 1978.
26. WEISS L. A pathobiologic overview of metastasis. Semin Oncol 4:5-17, 1977.
27. HART IR. "Seed and soil" revisited: mechanisms of site-specific metastasis. Cancer Metab Rev 1:5-17, 1982.
28. WEISS L. Metastasis: differences between cancer cells in primary and secondary tumors. Pathobiol Annu 10:51-81, 1980.
29. FIDLER IJ. Metastasis: quantitative analysis of distribution and fate of tumor cell emboli labelled with ^{125}I-5-iododeoxyuridine. JNCI 45:773-782, 1970.
30. POSTE G, TZENG J, KOESTLER T, ET AL. Interactions between tumor cell subpopulations in malignant tumors. *In* Cancer Invasion and Metastasis: Biologic and Therapeutic Aspects (Nicolson G, and Milas L, eds). New York, Raven Press, 1983, in press.
31. REDDY AL, and FIALKOW PJ. Multicellular origin of fibrosarcomas in mice induced by the chemical carcinogen 3-methylcholanthrene. J Exp Med 150:878-886, 1980.
32. HSU SH, LUK GD, KRUSH AJ, ET AL. Multiclonal origin of polyps in Gardner syndrome. Science 221:951-953, 1983.
33. NOWELL PS. The clonal evaluation of tumor cell subpopulations. Science 194:23-28, 1976.
34. FOULDS L. The experimental study of tumor progression: a review. Cancer Res 14:327-339, 1954.
35. CIFONE MA, and FIDLER IJ. Increasing metastatic potential is associated with increasing genetic instability of clones isolated from murine neoplasms. Proc Natl Acad Sci USA 78:6949-6952, 1982.
36. BOSSLET K, and SCHIRRMACHER V. High frequency generation of new immunoresistant tumor variants during metastasis of a cloned murine tumor line (ESb). Int J Cancer 29:195-202, 1982.
37. HARRIS JF, CHAMBERS AF, HILL RP, ET AL. Metastatic variants are generated spontaneously at a high rate in mouse KHT tumor. Proc Natl Acad Sci USA 79:5547-5551, 1982.
38. BOON T, SNICK JV, PEL AV, ET AL. Immunogenic variants obtained by mutagenesis of mouse mastocytoma P815. II T lymphocyte mediated cytolysis. J Exp Med 152:1184-1193, 1980.
39. FISHER MS, and CIFONE MA. Enhanced metastatic potential of murine fibrosarcomas treated in vitro with ultraviolet radiation. Cancer Res 41:3018-3023, 1981.
40. TALMADGE JE, WOLMAN SR, and FIDLER IJ. Evidence for the clonal origin of spontaneous metastases. Science 217:361-363, 1982.
41. POSTE G, TZENG J, DOLL J, ET AL. Evolution of tumor cell heterogeneity during progressive growth of individual lung metastases. Proc Natl Acad Sci USA 79:6574-6578, 1982.
42. HEPPNER GJ, and MILLER BE. Tumor heterogeneity: biological implications and therapeutic consequences. Cancer Metab Rev, 1983, in press.
43. POSTE G, DOLL J, and FIDLER IJ. Interactions between clonal subpopulations affect the stability of the metastatic phenotype in polyclonal populations of the B16 melanoma cells. Proc Natl Acad Sci USA 78:6226-6230, 1981.
44. SCHABEL FM, JR, GRISWOLD DP, CORBETT RH, ET AL. Testing therapeutic hypotheses in mice and men: observations on the therapeutic activity against advanced solid tumors of mice treated with anticancer drugs that have demonstrated or have potential clinical utility for treatment of advanced solid tumors of man. Methods Cancer Res 17:3-40, 1979.
45. SLACK NH, and BROSS IDJ. The influence of site of metastasis on tumor growth and response to chemotherapy. Br J Cancer 32:78-86, 1978.
46. FUGMANN RA, ANDERSON JC, STOLI R, ET AL. Comparison of adjuvant chemotherapeutic activity against primary and metastatic spontaneous murine tumors. Cancer Res 37:496-500, 1977.
47. TSURUO T, and FIDLER IJ. Differences in drug sensitivity among tumor cells from parental tumors, selected variants and spontaneous metastases. Cancer Res 41:3058-3064, 1981.
48. DONELLI MG, COLOMBO T, BROGGINI M, ET AL. Differential distribution of antitumor agents in primary and secondary tumors. Cancer Treat Rep 61:1319-1324, 1977.
49. SHAMI J, and WOLF W. A model for prediction of chemotherapy response to 5-fluorouracil based on the differential distribution of 5′ ^{18}F fluorouracil in sensitive vs resistant lymphocytic leukemia in mice. Cancer Res 37:2306-2308, 1977.
50. FIDLER IJ, BERENDT MJ, and OLDHAM RK. The rationale for and design of screening assays for the assessment of biological response modifiers for cancer treatment. J Biol Response Modifiers 1:15-26, 1982.
51. FIDLER IJ, and POSTE G. Macrophage-mediated destruction of malignant tumor cells and new strategies for the therapy of metastatic disease. Springer Semin Immunopathol 5:161-174, 1982.

Induced Cell Differentiation in Cancer Therapy[1]

Alexander Bloch[2,*]

Cancer can be considered a disorder of cell differentiation (1,2), a viewpoint that is most readily illustrated by the hematologic neoplasms. As schematically represented in figure 1, normal hematopoiesis proceeds from an immature stem cell, by a series of recognizable differentiation steps, to structurally and functionally mature cells that generally lack the capacity for further proliferation. Oncogenic conversion, defined as the arrest of differentiation without loss of proliferative capacity, can occur at any of the intermediate maturation steps. Because the maturation-arrested cells continue to proliferate, a population of immature "cancer" cells emerges and gives rise to adverse clinical manifestations.

Under normal conditions, proliferation and maturation are regulated by specific proteins produced by hematopoietic cells at various stages of development, as well as by auxiliary organs such as thymus and spleen (3,4). Such growth factors (GF) and differentiation factors (DF) respectively induce their target cells to proliferate or to differentiate. The lack of maturation progression that is characteristic of the neoplastic cell may thus be envisioned to derive from events that alter the cell's sensitivity to, or its dependence on, the presence of such factors. These changes may entail increased sensitivity to GF, decreased responsiveness to DF, decreased elaboration of DF by the host, and endogenous production of GF by the neoplastic cell itself (5).

Numerous studies have shown that human leukemic cell lines, primarily of myeloid lineage, retain their ability to differentiate in vitro when treated with natural DF present in conditioned media (6-13) or when exposed to a variety of chemical compounds that include the clinically active anticancer agents (14-20), natural effectors such as retinoic acid (21-23), certain corticosteroids including dexamethasone and prednisolone, and some prostaglandins, particularly of the E series (14). These cells are also induced to differentiate by phorbol esters such as tetradecanoyl phorbol acetate (TPA) (20,24-27), and by some polar substances including dimethyl sulfoxide (20,28-30). Cells of the lymphoid lineage can also undergo limited differentiation after they are exposed to TPA (31-35).

These observations clearly have relevance to the clinical setting. Almost all clinically effective anticancer agents are inhibitors of DNA synthesis or transcription (36); inhibitors of RNA or protein synthesis lack selectivity. If mere cytotoxicity were a sufficient condition for the long-term selective inhibition of tumor cell growth, then at least some clinically active agents should have emerged from the multitude of cytotoxic agents that in-

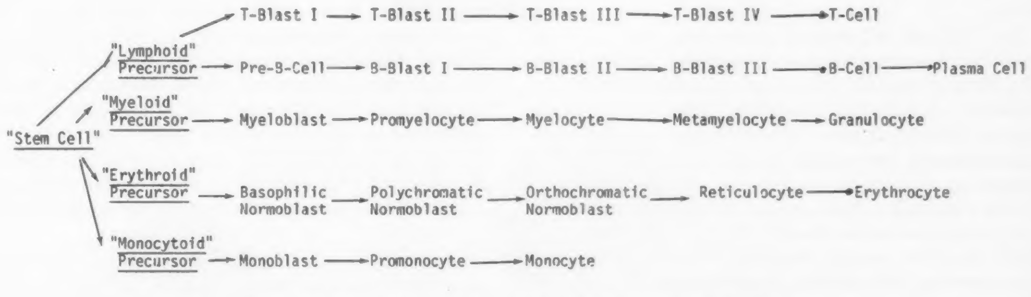

T-Blast I
T-Blast II
T-Blast III
T-Blast IV
T-Cell
"Lymphoid" Precursor
Pre-B-Cell
B-Blast I
B-Blast II
B-Blast III
B-Cell
Plasma Cell
"Myeloid" Precursor
Myeloblast
Promyelocyte
Myelocyte
Metamyelocyte
Granulocyte
"Stem Cell"
"Erythroid" Precursor
Basophilic Normoblast
Polychromatic Normoblast
Orthochromatic Normoblast
Reticulocyte
Erythrocyte
"Monocytoid" Precursor
Monoblast
Promonocyte
Monocyte

FIGURE 1.—Scheme of hematopoietic cell differentiation (modified from ref 72).

---

[1] Supported in part by Public Health Service grant CA-12585 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[2] Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo.

[*] *Reprint requests to:* Alexander Bloch, PhD, Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, 666 Elm St, Buffalo, NY 14263.

terfere with RNA or protein synthesis. The fact that this is not the case supports the notion that selective antitumor action depends to a significant extent on the specific inhibition of DNA-centered activities. This selectivity is envisioned to derive, in part, from the ability of DNA-targeted agents to induce the maturation of sensitive cancer cells past the stage of oncogenic differentiation arrest, thereby removing their capacity for unlimited proliferation.

This notion is supported by experimental data derived from in vitro studies. Only DNA-specific inhibitors such as cytarabine or daunorubicin are capable of effectively inducing the differentiation of various myeloid leukemic cell lines (37), whereas inhibitors of RNA or protein synthesis, including cordycepin and puromycin, are unable to achieve this result. Differentiation induced with anticancer agents or with phorbol esters entails the conversion of leukemic myeloblasts (20) or promyelocytes (16-19,24-27) to cells with morphologic characteristics of monocytes or macrophages (fig 2b), whereas treatment with dimethyl sulfoxide or retinoic acid stimulates maturation along the granulocyte path (fig 2c) (22,23, 28-30). At effective concentrations, maturation is initiated by TPA and by retinoic acid without significant loss of cell viability, whereas the DNA-specific drugs can be cytocidal to a fraction of the leukemic target cell population (20). Since differentiation proceeds from a stage in G_1 (38), cells that at the time of drug administration are present in another phase of the cell cycle, where they are optimally sensitive to the cytocidal action of the drug (eg, S phase for cytarabine), appear to be the ones lethally damaged. Cells that are present in less sensitive phases, or that enter the sensitive phase when the drug concentration is already diminished, may proceed to differentiation in G_1. The degree of cytotoxicity that a DNA-specific agent produces depends largely upon the concentration of DF present in the medium. If DF levels are adequate, differentiation can be effectively induced by minimally toxic or by nontoxic concentrations of DNA-reactive drugs.

An "optimal differentiation-inducing dose" can be specified, which defines the concentration of a drug that, at a given level of DF, causes optimal induction of differentiation at minimal cytotoxicity. This dose varies in amount, depending upon the pharmacokinetic properties of a compound; its cycle specificity; and the rate of its intracellular accumulation, activation, and degradation. It is also a function of the degree of synchrony of the target cell population and the fraction of tumor cells that are in cycle. Since the crucial characteristic of the differentiation-inducing DNA-directed agents would appear to be their ability to interfere with the elaboration of proliferation-associated messages without impairing the subsequent transcription of differentiation-associated information (fig 3), the effective intracellular drug concentration may have to peak before the differentiation program is transcribed. To allow the transition from proliferation to differentiation, the drug must not impede RNA and protein synthesis. However, a partial or delayed inhibition of these processes appears to be compatible with the expression of differentiation-associated events, even if these do not proceed to terminal maturation (35). What is required is the interruption of the oncogenically induced freeze that holds the neoplastic cells in their proliferation mode. A temporary interruption of proliferation, based on cytotoxic mechanisms of drug action, would not achieve this objective, since the oncogenic defect would again be expressed after resumption of proliferation.[3] In the same vein, maximal tumor cell kill can be obtained only at higher drug concentrations leading to decreased selectivity.

The molecular mechanisms that are proposed to underlie the induced differentiation events are summarized schematically in figure 3. The primary event responsible for freezing the neoplastic cell in its proliferation mode is held to be the accumulation of a proliferation factor or factors (PF) at a sufficiently high concentration and in properly modified form. This factor maintains proliferation and may correspond to the labile protein previously advocated (39) to be required for cell-cycle progression from G_1 to S. It may relate to cellular oncogene products that cause oncogenic conversion by freezing the neoplastic cell in the proliferation mode. Expression of oncogenes is decreased in differentiated cells (40), and in ML-1 myeloblastic leukemia cells, a significant decline in c-myb oncogene expression is detected within 3 hours after addition of the differentiation inducer (41). The PF-mediated activity appears to be subject to regulation by specific exogenous signals, including GF and DF. GF may act by stabilizing PF (42), possibly through the intervention of GF-associated tyrosine-specific protein kinases (43,44). Some oncogene products appear to be functionally (45) and structurally (46) related to GF that stimulate tyrosine-specific kinases, whereas others display tyrosine-specific protein kinase activity themselves (47). Since both categories of kinases recognize some of the same substrate determinants (48,49), they are likely capable of maintaining the neoplastic cell in its proliferative state through similar or related mechanisms. At sufficiently high concentrations, DF are capable of interrupting the oncogene-maintained proliferation cycle (13), possibly by deactivating PF. According to this scheme, the ability of DNA-directed agents to induce neoplastic cell differentiation would derive from the curtailed tran-

---

[3] The antitumor activity of agents such as vincristine and L-asparaginase, which do not appear to interfere primarily with DNA synthesis or function, may derive from their cytotoxic action. That mechanism does not provide for long-term remissions. Depending on the nature of the target cells, the observed inhibition of DNA synthesis by vincristine, and the modification of the cell membrane by L-asparaginase might serve, however, as primary stimuli for differentiation, although such stimulation is not observed in the myeloblastic leukemia model.

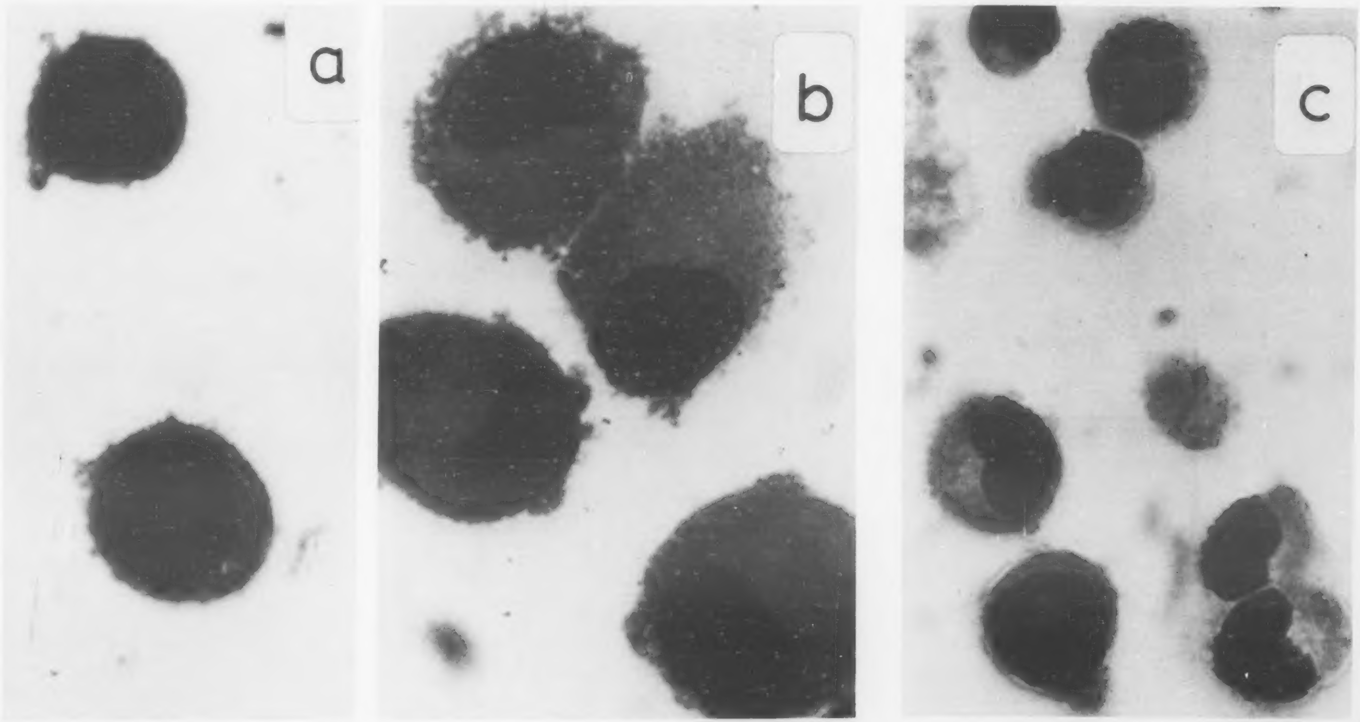


FIGURE 2.—Induced in vitro differentiation of ML-1 human myeloblastic leukemia cells (a) to monocyte/macrophages (b), or to granulocytes (c).

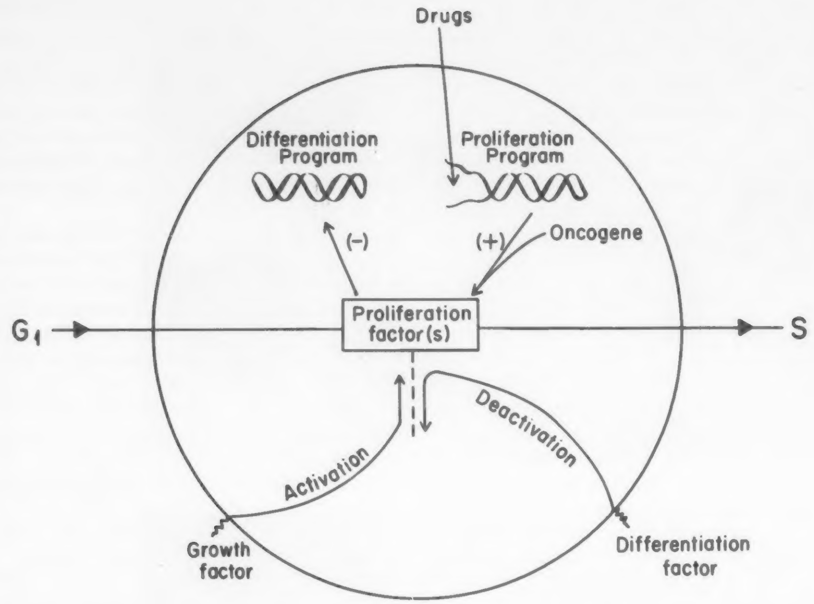


FIGURE 3.—Schematic outline of cellular events considered to participate in induced tumor cell differentiation.

scription of mRNA required for the synthesis of PF. Lowering of PF, probably in association with the action of natural DF, would then allow the expression of the differentiation-associated program.

Various experimental observations support the hypotheses outlined in this scheme. Certain phorbol esters, such as TPA, which inhibit binding of GF to specific receptors (50), or natural effectors, such as retinoic acid, which interfere with the mitogenic stimulation exerted by GF (51), are powerful inducers of leukemic cell differentiation. Some polar substances, such as dimethyl sulfoxide, have a similar effect (19,28-30), possibly exerted through their action on the lipoidal cell membrane. Increasing the level of DF causes the leukemic cells to undergo differentiation (13); treatment with agents such as dexamethasone or lipid A causes them to generate endogenous DF (52,53)

Since treatment with DNA-targeted agents is assumed to lower the concentration of PF, cells exposed to low doses of such agents should exhibit enhanced sensitivity to exogenously provided DF. This is indeed the case (54,55). Exposure of leukemic cells to an agent such as dactinomycin or daunorubicin, followed by the administration of conditioned-medium DF, at concentrations at which neither drug nor DF demonstrate significant differentiation-inducing ability, leads to extensive cell maturation. A similar result is obtained when drug and DF are applied simultaneously (56). This phenomenon of sensitization may in fact constitute a critical component of clinical drug therapy and provides a convincing rationale for the use of low-dose regimens at minimally toxic or nontoxic doses.

Some general conclusions can be drawn from the aforementioned considerations concerning the possible role of differentiation in cancer therapy. Primary among these conclusions is the recognition that the oncogenic arrest of normal cell differentiation is not necessarily an irrevocable event. This principle is not limited to leukemic cells, but applies to other types of neoplastic cells as well, including choriocarcinoma (15), embryonal carcinoma (57), and neuroblastoma (58,59). Their ability to undergo differentiation upon drug treatment in vitro appears to constitute a meaningful correlate of therapeutic response. Since solid tumors, such as colorectal carcinoma, also appear to evolve as a result of arrested differentiation (60,61), their unresponsiveness to current treatment may result from the large noncycling cell fraction (62) that is inaccessible to drug-induced lowering of PF. To be effective, low-dose regimens would need to be applied over periods of weeks or months, so that any tumor cells entering into cycle could be sensitized to the action of natural DF, assuming that these are produced at sufficiently high concentrations. Unlike cytotoxic regimens, such low-dose therapy is not likely to interfere with the continued elaboration of such factors by natural producer cells. Similar considerations apply to the treatment of slowly growing metastatic tumors as well.

The level of PF and DF present at a given stage in the disease process may constitute a crucial determinant of tumor progression. A decreased concentration of DF, resulting from the leukemia-associated imbalance in hematopoietic cell population, may enhance the disease process. Evidence has been adduced (63) which demonstrates that under the preleukemic conditions existing in myelodysplastic syndromes, the ability to generate DF is diminished. One of the potential benefits of differentiation-centered therapy is the observed ability of leukemic cells to produce normal regulatory factors during the process of their maturation (64).

Since leukemic cells are capable of producing factors that inhibit normal stem cell activity (65) and prevent differentiation (66), removal of the tumor cell burden would seem to constitute an important component of differentiation-centered therapy. The manner in which this reduction is brought about needs to be carefully selected, since overtly cytotoxic regimens are likely to impair the production of DF. Indeed, the sometimes observed appearance of neoplasms following immunosuppression may well derive from deficiencies in the production of DF. The use of natural DF, obtained through molecular cloning, would clearly add a further dimension to cancer therapy.

Sensitization of neoplastic cells with low doses of DNA-directed drugs, followed by the application of natural DF or of agents that stimulate their production, should result in a therapeutic benefit exceeding that obtainable with either component alone. Agents that have the capacity to enhance the formation of DF by some producer cells include lipopolysaccharides and poly(I).poly(C) (67). Certain corticosteroids have the capacity to stimulate the endogenous formation of DF (52). That does not mean that single-component therapy cannot be effective, particularly where the supply of natural DF is adequate. Recent reports (68–70) have demonstrated the induction of complete remissions in several patients with acute myelogenous leukemia treated for various periods of time with low doses of cytarabine. Such therapy may rely on the presence of natural DF, and determining the levels at which these are present can aid the selection of the most suitable regimen to be applied. Appropriate human cell lines, cultured in vitro, can serve as suitable test systems for this purpose.

Since agents that interfere with GF binding or activity are effective inducers of differentiation, increased emphasis on their development for therapeutic use appears indicated. Among these are antibodies directed against specific GF or their receptors. TPA, because of its well-established promoter activity, does not readily qualify for this task, but nonpromoting analogs and other agents that interfere with GF action possess greater therapeutic potential. In fact, interference with the specific action of GF may be of use not only for therapy but also for prevention. The chemopreventive utility of retinoic acid (71) may well derive from its ability to effectively stimulate cell differentiation.

In conclusion, a differentiation-oriented therapeutic approach can draw upon various modalities including the use of noncytotoxic or minimally cytotoxic doses of DNA-specific agents capable of sensitizing the tumor cells to the action of natural DF; the employment of agents that stimulate the formation of endogenous DF; the administration of natural DF and the stimulation of their formation by producer cells; the application of agents, including antibodies, that prevent the action of specific GF, allowing existing DF to become active; and the use of compounds that mimic DF in their maturation-inducing ability.

Such an approach has significant potential for making a tangible contribution to the therapy of cancer.

REFERENCES

1. PIERCE G, SHILES R, and FINK L. Cancer, a Problem of Developmental Biology. Englewood Cliffs, Prentice Hall, 1978.
2. GREAVES MP. "Target" cells, cellular phenotypes, and lineage fidelity in human leukemia. J Cell Physiol 1:113–125, 1982.
3. MAK TW, and MCCULLOCH EA. Cellular and Molecular Biology of Hemopoietic Stem Cell Differentiation. New York, AR Liss, 1982.
4. CLARKSON B, MARKS PA, and TILL JE. Differentiation of normal and neoplastic hematopoietic cells. Cold Spring Harbor Laboratory, 1978.
5. TODARO GL. Growth factors and their receptors in normal and transformed cells. Fed Proc 41:2987, 1982.
6. MAEDA M, and ICHIKAVA Y. Production of growth and differentiation-stimulating factors for mouse leukemia cells by different cell species. Gann 64:257–263, 1973.
7. TILL JE, and MCCULLOCH EA. Hemopoietic stem cell differentiation. Biochim Biophys Acta 605:431–459, 1980.
8. YAMAMOTO Y, TOMIDA M, and HOZUMI M. Production by mouse spleen cells of factors stimulating differentiation of mouse myeloid leukemic cells that differ from the colony-stimulating factor. Cancer Res 40:4804–4809, 1980.
9. METCALF D. Sources and biology of regulatory factors active on mouse myeloid leukemia cells. J Cell Physiol 1:175–182, 1982.
10. MOORE MAS. G-CSF: its relationship to leukemia differentiation-inducing activity and other hemopoietic regulators. J Cell Physiol 1:53–64, 1982.
11. SACHS L. Control of growth and normal differentiation in leukemia cells: regulation of the developmental program and restoration of the normal phenotype in myeloid leukemia. J Cell Physiol 1:151–164, 1982.
12. ISCOVE NN, ROITSCH CA, WILLIAMS N, ET AL. Molecules stimulating early red cell, granulocytes, macrophage and megakaryocyte precursors in culture: similarity in size, hydrophobicity, and cleavage. J Cell Physiol 1:65–78, 1982.
13. TAKEDA K, MINOWADA J, and BLOCH A. Differential ability of mitogen-stimulated human leukocyte-conditioned media to induce F_c receptors in human leukemia cells. Cell Immunol 79:288–297, 1983.
14. HOZUMI M, HONMA V, TOMIDA M, ET AL. Induction of differentiation of myeloid leukemia cells with various chemicals. Acta Haematol Japn 42:941–952, 1979
15. FRIEDMAN SJ, and SKEHAN P. Morphological differentiation of human choriocarcinoma cells induced by methotrexate. Cancer Res 39:1960–1967, 1979.
16. COLLINS SJ, BODNER A, TING R, ET AL. Induction of morphological and functional differentiation of human promyelocytic leukemia cells (HL-60) by compounds which induce differentiation of murine leuke-

mia cells. Int J Cancer 25:213-218, 1980.

17. LOTEM J, and SACHS L. Potential pre-screening for therapeutic agents that induce differentiation in human myeloid leukemia cells. Int J Cancer 25:561-564, 1980.
18. PAPAC RJ, BROWN AE, SCHWARTZ EL, ET AL. Differentiation of human promyelocytic leukemia cells in vitro by 6-thioguanine. Cancer Lett 10:33-38, 1980.
19. BODNAR AJ, TING RC, and GALLO RC. Induction of differentiation of human promyelocytic leukemia cells (HL-60) by nucleosides and methotrexate. JNCI 67:1025-1030, 1981.
20. TAKEDA K, MINOWADA J, and BLOCH A. Kinetics of appearance of differentiation-associated characteristics in ML-1, a line of human myeloblastic leukemia cells, after treatment with 12-*O*-tetradecanoylphorbol-13-acetate, dimethyl sulfoxide, or 1-β-D-arabinofuranosylcytosine. Cancer Res 42:5152-5158, 1982.
21. STRICKLAND S, and MAHDANI V. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. Cell 15:393-403, 1978.
22. BREITMAN TR, SELONICK SE, and COLLINS SJ. Induction of differentiation of the human promyelocytic leukemia cell lines (HL-60) by retinoic acid. Proc Natl Acad Sci USA 77:2936-2940, 1980.
23. HONMA Y, TAKENAGA K, KASUKABE T, ET AL. Induction of differentiation of cultured human promyelocytic leukemia cells by retinoids. Biochem Biophys Res Commun 95:507-512, 1980.
24. ROVERA G, O'BRIEN TG, and DIAMOND L. Induction of differentiation in human promyelocytic leukemia cells by tumor promoters. Science 204:868-870, 1979.
25. HUBERMAN E, and CALLAHAM MF. Induction of terminal differentiation in human promyelocytic leukemia cells by tumor-promoting agents. Proc Natl Acad Sci USA 76:1293-1297, 1979.
26. LOTEM J, and SACHS L. Regulation of normal differentiation in mouse and human myeloid leukemic cells by phorbol esters and the mechanism of tumor promotion. Proc Natl Acad Sci USA 76:5158-5162, 1979.
27. KOEFFLER HP, BAR-ELI M, and TERRITO MC. Phorbol ester effect on differentiation of human myeloid leukemia cell lines blocked at different stages of maturation. Cancer Res 41:919-926, 1981.
28. FRIEND C, SCHER W, HOLLAND JG, ET AL. Hemoglobin synthesis in murine virus induced leukemic cells in vitro: stimulation of erythroid differentiation by dimethyl sulfoxide. Proc Natl Acad Sci USA 68:378-382, 1971.
29. TANAKA M, LEVY J, TERADA M, ET AL. Induction of erythroid differentiation in murine virus infected erythroleukemic cells by highly polar compounds. Proc Natl Acad Sci USA 72:1003-1006, 1975.
30. COLLINS SJ, RUSCETTI FW, GALLAGHER RE, ET AL. Terminal differentiation of human promyelocytic leukemia cells induced by dimethylsulfoxide and other polar compounds. Proc Natl Acad Sci USA 75:2458-2462, 1978.
31. NAGASAWA K, and MAK TW. Phorbol esters induce differentiation in human malignant T lymphoblasts. Proc Natl Acad Sci USA 77:2964, 1980.
32. LEBIEN T, KERSEY J, NAKAZAWA S, ET AL. Analysis of human leukemia/lymphoma cell lines with monoclonal antibodies BA-1, BA-2, and BA-3. Leukemia Res 6:299, 1982.
33. NAKAO Y, MATSUDA S, FUJITA T, ET AL. Phorbol ester-induced differentiation of human T-lymphoblastic cell line HPB-ALL. Cancer Res 42:3843-3850, 1982.
34. TOTTERMAN TH, NILSSON K, and SUNDSTROM C. Phorbol ester-induced differentiation of chronic lymphocytic leukemia cells. Nature 288:176-178, 1980.
35. SAKAGAMI H, MINOWADA J, OZER H, ET AL. Induction of differentiation in human cultured non-T, non-B cell lines by 12-*O*-tetradecanoyl phorbol 13-acetate. Leukemia Res, in press.
36. BLOCH A. Purine and pyrimidine analogs in cancer chemotherapy. *In* New Leads in Cancer Therapeutics (Mihich E, ed). Boston, GK Hall and Co, 1981, pp 65-72.
37. TAKEDA K, MINOWADA J, LEASURE JA, ET AL. Comparison of the ability of various cytotoxic agents to induce the differentiation of human myeloblastic leukemia cells in vitro. Proc Am Assoc Cancer Res 23:226, 1982.
38. WILLIE JJ, JR, and SCOTT RE. Topography of the predifferentiation G_D growth arrest state relative to other growth arrest states in the G_1 phase of the cell cycle. J Cell Physiol 112:115-122, 1982.
39. MEDRANO EE, ARANYI P, and PARDEE AB. On the regulation of eukaryotic cell growth: An approach to the behavior of normal and transformed cells. *In* Cellular Responses to Molecular Modulators (Mozes LW, Schultz J, Scott WP, et al, eds). New York, Academic Press, 1981, pp 49-51.
40. WESTIN EH, WONG-STAAL F, GEMMANN EP, ET AL. Expression of cellular homologues of retroviral *onc* genes in human hematopoietic cells. Proc Natl Acad Sci USA 79:2490-2494, 1982.
41. CRAIG RW, and BLOCH A. Early decline in c-myb oncogene expression in the differentiation of human myeloblastic leukemia (ML-1) cells induced with 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Res, in press.
42. BALLARD FJ, KNOWLES SE, WONG SC, ET AL. Inhibition of protein breakdown in cultured cells is a consistent response to growth factors. FEBS Lett 144:209-212, 1980.
43. COHEN S, CARPENTER G, and KING L, JR. Epidermal growth factor-receptor-protein kinase interactions. J Biol Chem 255:4834-4842, 1980.
44. USHIRO H, and COHEN S. Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membrane. J Biol Chem 255:8363-8365, 1980.
45. TODARO GJ, DELARCO JE, and COHEN S. Transformation by murine and feline sarcoma viruses specifically blocks binding of epidermal growth factor to cells. Nature 264:26-31, 1976.
46. DOOLITTLE RF, HUNKAPILLER MW, AARONSON SA, ET AL. Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221:275-277, 1983.
47. ERIKSON RL, PURCHIO AE, ERIKSON E, ET AL. Molecular events in cells transformed by rous sarcoma virus. J Cell Biol 87:319-325, 1980.
48. KUDLOW JE, BUSS JE, and GILL GN. Anti-pp60^{src} antibodies are substrates for EGF-stimulated protein kinase. Nature 290:519-521, 1981.
49. CHINKERS M, and COHEN S. Purified EGF receptor-kinase interacts specifically with antibodies to Rous sarcoma virus transforming protein. Nature 290:516-519, 1981.
50. LEE L-S, and WEINSTEIN IB. Tumor-promoting phorbol esters inhibit binding of epidermal growth factor to cellular receptors. Science 202:314-315, 1978.
51. JETTEN AM. Effects of retinoic acid on the binding and mitogenic activity of epidermal growth factor. J Cell Physiol 110:235-240, 1982.
52. HONMA V, KASUKABE T, and HOZUMI M. Production of differentiation-stimulating factor in cultured mouse myeloid leukemia cells treated by glucocorticoids. Exp Cell Res 111:261-267, 1978.
53. WEISS B, and SACHS L. Indirect induction of differentiation in myeloid leukemic cells by lipid A. Proc Natl Acad Sci USA 75:1374-1378, 1978.
54. HAYASHI M, OKABE J, and HOZUMI M. Sensitization of resistant myeloid leukemia cells by anticancer drugs to factor-stimulating differentiation. Gann 70:235-238, 1979.
55. OKABE J, HONMA Y, HAYASHI M. ET AL. Actinomycin D restores in vivo sensitivity to differentiation induction of non-differentiating mouse myeloid leukemia cells. Int J Cancer 24:87-91, 1979.
56. CRAIG RW, FRANKFURT O, SAKAGAMI H, ET AL. In preparation.
57. STRICKLAND S, and SAWEY MJ. Studies on the effect of retinoids on the differentiation of teratocarcinoma stem cells in vitro and in vivo. Dev Biol 78:76-85, 1980.
58. SCHUBERT D, and JACOB F. 5-Bromodeoxyuridine-induced differentiation of a neuroblastoma. Proc Natl Acad Sci USA 67:247-254, 1970.
59. SILBERT SW, and GOLDSTEIN MN. Drug-induced differentiation of a rat glioma in vitro. Cancer Res 32:1422-1427, 1972.
60. LIPKIN M, BLATTNER WE, FRAUMENI J, JR, ET AL. Tritiated thymidine labeling distribution as a marker for hereditary predisposition to colon cancer. Cancer Res 43:1899-1904, 1983.
61. LIPKIN M. Proliferation and differentiation of gastrointestinal cells

in normal and disease states. *In* Physiology of the Gastrointestinal Tract (Johnson L, ed). New York, Raven Press, 1981, pp 145-167.

62. WEISBURGER JH, REDDY BS, and JOFTES DL. Colo-rectal cancer report No. 19. Geneva, UICC, 1975, pp 69-76.
63. KALRA A, SAKAGAMI H, HIGBY D, ET AL. In preparation.
64. MAEDA M, and ICHIKAWA Y. Production of a colony-stimulating factor following differentiation of leukemic myeloblasts to macrophages. J Cell Physiol 102:323-331, 1980.
65. BROXMEYER HE, JACOBSEN N, KURLAND J, ET AL. In vitro suppression of normal granulocytic stem cells by inhibitory activity derived from human leukemia cells. JNCI 60:497-510, 1978.
66. OKABE J, HAYASHI M, HONMA Y, ET AL. Differentiation of mouse myeloid leukemia cells is inhibited by a factor from non-differentiating leukemia cells. Int J Cancer 22:570-575, 1978.
67. YAMAMOTO Y, TOMIDA M, HOZUMI M, ET AL. Enhancement by immunostimulants of the production by mouse spleen cells of factor(s) stimulating differentiation of mouse myeloid leukemic cells. Gann 72:828-833, 1981.
68. BACCARANI M, and TURA S. Differentiation of myeloid leukemic cells: new possibilities for therapy. Br J Haematol 42:485-490, 1979.
69. HOUSETT M, DANIEL MT, and DEGOS L. Small doses of ara-C in the treatment of acute myeloid leukemia: differentiation of myeloid leukemia cells. Br J Haematol 51:125-129, 1982.
70. MALONEY WC, and ROSENTHAL DS. Treatment of early acute nonlymphatic leukemia with low dose cytosine arabinoside. *In* Modern Trends in Human Leukemia (Neth R, Gallo RC, Graf T, et al, eds). Berlin, Springer, pp 59-62.
71. BOLLAG W, and MATTER A. From vitamin A to retinoids in experimental and clinical oncology: achievements, failures and outlook. Ann NY Acad Sci 359:9-24, 1981.
72. TAKEDA K, MINOWADA J, and BLOCH A. The role of drug-induced differentiation in the control of tumor growth. *In* Proceedings of the 9th International Symposium on the Biologic Characterization of Human Tumors. Bologna, 1981. Berlin, Akademie Verlag, 1983, pp 275-281.

Current Condition and Prognosis of Tumor Immunotherapy: A Second Opinion[1]

Michael J. Mastrangelo,[2,3,*] David Berd,[2,4] and Henry C. Maguire, Jr[2,5]

That manipulation of the immune system might result in the regression of malignant tumors is a compelling idea that has stimulated scientific experimentation for > 80 years. At the turn of the century, Coley (1) attempted to stimulate resistance to human tumors by the administration of bacteria and bacterial toxins. In the 1940s, Gross (2) discovered that mice could be immunized against a tumor that developed in a mouse of the same inbred strain. At the time, this work received little notice. In the 1950s, Prehn and Main (3) demonstrated the existence of tumor-specific transplantation antigens on chemically induced murine neoplasms. By 1970, some daring investigators (4-6) were using immunomodulating agents to treat human cancers. By 1980, the results of hundreds of clinical immunotherapy trials had been published. Yet, in 1984, the only fair and accurate evaluation of human cancer immunotherapy is that its efficacy is unproven and uncertain. Currently, with the possible exception of the injection of BCG into dermal metastases of malignant melanoma, immunotherapy has no place in the routine treatment of patients with cancer.

Is cancer immunology just another one of those fascinating but mistaken ideas which, like the homunculus theory in genetics and the phlogiston theory in physics, is deserving of nothing more than a few lines in a textbook on the history of science? Or have we expected "too much, too soon" from tumor immunology and has its clinical application been hasty and premature?

In this review, we will attempt to support the second of these two alternatives. We hope to convince the reader that the concept of tumor-induced immune responses is sound, that some of the major conclusions drawn by investigators in this area may not be correct, and that a better understanding of basic immunology will lead to more successful application of tumor immunology to clinical medicine. This is, in essence, a second opinion on the current condition and prognosis of tumor immunotherapy. As such, it is not an exhaustive review of the extant literature, but rather focuses on material germane to several of the fundamental questions confronting tumor immunotherapists.

We will address in order the following six questions. (*a*) Are there tumor-specific cell surface antigens on human tumors? (*b*) Are highly immunogenic animal tumors relevant models for human immunotherapy? (*c*) What is the relative importance (and clinical promise) of cellular versus humoral tumor-specific immunity? (*d*) Does tumor burden represent a critical limitation to immunotherapy? (*e*) Is nonspecific cytotoxicity relevant to the rejection of tumors? (*f*) Is the presence of tumor-specific suppressor cells a serious impediment to successful immunotherapy?

Are There Tumor-Specific Cell Surface Antigens on Human Tumors?

The demonstration of tumor-specific antigens in animal systems relied heavily on transplant rejection experiments in syngeneic animals. Prehn and Main (3) convincingly demonstrated that a mouse that had been previously immunized by growth and excision of a tumor could reject a transplant of the same chemically induced tumor, while accepting a skin transplant from the animal in which the tumor originally arose. With these techniques, antigens on chemically induced tumors seemed to be unique for each tumor. In contrast, similar experiments with virally induced tumors indicated that all of the tumors induced by the same virus had a common major surface antigen (7-10).

There has been a major debate as to whether these data generated with chemically and virally induced animal tumors imply the existence of similar antigens on spontaneous human tumors. Indeed, there are some who doubt the presence of comparable antigens on spontaneous human tumors (11). In the mouse models, the probability of a methylcholanthrene-induced tumor being immunogenic falls as the inducing concentration of chemical carcinogen falls and as the time between the introduction of carcinogen and appearance of tumor increases (12). In man,

---

[1] Supported by Public Health Service grant CA-32123 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by an appropriation from the Commonwealth of Pennsylvania.

[2] Fox Chase Cancer Center, Philadelphia, PA.

[3] Temple University School of Medicine, Philadelphia, PA.

[4] University of Pennsylvania School of Medicine, Philadelphia.

[5] Hahnemann University, Philadelphia, PA.

[*] *Reprint requests to:* Michael J. Mastrangelo, MD, Fox Chase Cancer Center, 7701 Burholme Ave, Philadelphia, PA 19111.

exposure is likely to be to a low concentration of carcinogen and the induction periods are generally conceded to be long. Virally induced animal tumors are generally strongly immunogenic. While it is widely assumed that viruses cause some human tumors, as Hewitt (11) points out, the suspect viruses "are not specifically tumorigenic, are sometimes responsible for pre-existing pathology on which neoplasia is superimposed and are often of very wide prevalence." He therefore finds it "inconceivable that the general run of laboratory virus tumors could ever be preferentially taken as valid models of the general run of clinical cancer."

The transplant rejection techniques used to demonstrate tumor-specific antigens on animal tumors are poorly suited for use in man. Such studies, if done at all, must be limited to autotransplants in patients with incurable malignant disease. Several investigators have studied human cancer using this methodology. The most extensive of these studies was conducted by Southam and Beunschwig (13). Using cells from fresh autologous tumor specimens, these investigators demonstrated that inocula of 10^8 cells would almost always grow, whereas only 50% takes were noted with inocula of 10^6 cells and no growth was noted with inocula of 10^4 tumor cells. These studies suggest some measure of host resistance, even in patients with incurable, progressive disease. Whether this resistance to tumor growth was immunologic in origin could not be determined. The autotransplantation techniques are of limited usefulness in the detection of tumor-specific antigens in man.

To circumvent the limited applicability of transplant rejection techniques in man, in vitro assays were developed to demonstrate tumor-specific antigens. The tumor type most extensively studied with these techniques has been malignant melanoma. The data from these assays have been extensively reviewed in an attempt to determine if they supported the existence of tumor antigens and a host immune response to these antigens (14). The results were often conflicting, controls absent or inappropriate, and the design inadequate to distinguish tumor-associated antigens from other antigens.

Serology has proven to be the most sensitive of the in vitro techniques for analyzing the surface composition of tumor cells. Oettgen (15) has analyzed sera from 75 patients with melanoma for antibody against autologous and allogeneic melanoma cell lines. Only four of 75 patients had antibody directed against determinants restricted to their own cultured tumor cells. An additional five patients had antibody against determinants shared by allogeneic tumors of similar and, in some cases, dissimilar origins. Twenty-one patients had antibody directed against more widely distributed antigens. These data constitute the most convincing body of evidence for the existence of immunogenic melanoma-associated antigens. However, the prevalence of reactive antigens that are restricted to tumor cells is low. The possible explanations for this are several and include the nonexpression or low expression of tumor-specific antigens. Also, although the cell may express tumor-specific antigens, these antigens may be masked by "self" antigens and thus go unrecognized (16).

To further explore the melanoma cell surface for tumor-specific markers, a series of murine monoclonal antibodies has been developed. A least 20 different proteins in addition to HLA antigens have been found on melanoma cells by use of these monoclonal antibodies (17). Some of these proteins, such as gp 150, are found on a variety of both malignant and nonmalignant cells. Others, such as p 97 and gp 95, are restricted to cells from melanomas, astrocytomas, and possibly normal brain. Reisfeld et al (18) have described a monoclonal antibody (9.2.27) which binds to all melanoma cell lines tested but not to carcinoma, fibroblast, or lymphoid cell lines. This antibody also binds to melanoma cells dissociated from most freshly excised melanoma metastases, but not to cells from normal moles, benign or malignant breast tumors, colon carcinoma, or Hodgkin's disease. The "antigen" against which this monoclonal antibody is directed is a 250-kd glycoprotein.

Thus, there is seemingly one or a number of melanoma cell surface proteins that are "unusual" (ie, preferentially expressed on cancer cells in the adult host) and, thus, potentially exploitable as inducers of tumor-specific immunity or as targets of passive/adoptive immunotherapy. Are the counterparts of the classic tumor-specific transplantation antigens, that are responsible for tumor rejection in animal systems, included among these "unusual" surface proteins? Perhaps not. With rare exceptions (19), even the most immunogenic of the transplant rejection antigen (which has been prematurely applied), they must be demonstrated to be immunogenic in their host. As noted above, there are few data that show naturally antigen (which has been prematurely applied), they must be demonstrated to be immunogeneic in their host. As noted above, there are few data that show naturally occurring antibody in tumor-bearing patients, and Oettgen (15) has been unable to demonstrate in any but a few instances an antibody response to immunization with an autologous tumor vaccine. Investigation of cell-mediated immune responses has been hampered by technical problems. The in vitro data obtained from lymphocyte-mediated cytotoxicity assays and the autologous mixed lymphocyte tumor cell assay are inconclusive (14,21). These difficulties in demonstrating immune responses to human tumor cell surface-associated proteins and to spontaneous animal tumors may be related to host suppressor cell function. This subject will be addressed in a subsequent section.

In summary, 27 years after the publication of the classic experiments of Prehn and Main (3), it has still not been demonstrated with certainty that "spontaneous" human malignancies bear "antigenic" determinants not found on their nonmalignant counterparts. Perhaps the presence of a unique antigen on a tumor cell surface is not a requirement for its immunologic rejection and the presence of an "unusual" antigen may be sufficient. Monoclonal antibody studies have identified several "unusual" proteins which are candidates for the designation of tumor antigen if immunogenicity can be demonstrated. The isolation and xenogenization of these proteins, as well as efforts to abrogate suppressor T-cell function, seem to be promising approaches for transforming these tumor-associated proteins to functionally specific human tumor-associated antigens.

Are Highly Immunogenic Animal Tumors Relevant Models for Human Tumor Immunotherapy?

It is our contention that if tumor immunotherapy can be effective, this will be most readily demonstrable in animal models. In a large number of animal models, tumor antigens have been conclusively demonstrated on the surface of tumor cells. Outbred and syngeneic animals bearing primary or transplantable tumors are available in relative abundance and can be studied in ways not possible in a clinical setting. However, there has been a long and lively debate regarding the propriety of using strongly immunogenic animal tumors as models for human immunotherapy (11,22-27). It is obvious that these animal models are not entirely analogous to the human situation, in that the tumors are most often not spontaneous and are generally rapidly growing. The recipients of transplanted animal tumors usually are otherwise normal. Nonetheless, it seems quite reasonable to assume that if immunotherapy is not successful in these contrived (eg, immunogenicity) and controlled (eg, tumor load) animal tumor models, the chance for success with human tumors is practically nil. A previous comparison of the results achieved with tumor immunotherapy in animals and in man showed a good correlation, albeit perhaps by coincidence (28). Some examples are given in the sections that follow. It seems unnecessary to further debate this issue, since there is yet to be reported a meaningful antitumor effect demonstrable in animals that has not been shown to be reproducible in man.

What Is the Relative Importance (and Clinical Promise) of Cellular Versus Humoral Tumor-Specific Immunity?

Allograft rejection is most effectively mediated by T lymphocytes. Immunoprophylaxis studies suggest that tumor rejection also is most effectively accomplished via cellular mechanisms. However, newer developments in cellular and humoral immunity mandate that this subject be reconsidered. We will address this issue by assessing the accomplishments of immune serum or cells in immunoprophylaxis and immunotherapy trials in animals and in man.

Immunoprophylaxis With Cells or Serum

Many investigators have demonstrated that immune lymphocytes administered to animals prior to challenge with tumor cells are capable of preventing the subsequent outgrowth of tumor. This work has been reviewed extensively (29,30). Immunoprophylaxis with serum is more difficult to demonstrate, and when demonstrable, is less dramatic. Some successes have been noted. For example, Gorer and Amos (31) raised antibody to the EL-4 lymphoma in allogeneic mice and noted that the antibody could protect against tumor growth, if given prior to EL-4 injection. The unavailability of immune effector elements and vagaries regarding the timing of their administration in relation to tumor emergence have prevented the study of passive/adoptive immunoprophylaxis in man.

Immunotherapy With Serum

The subject of therapy via the passive administration of immune serum, prior to the availability of murine monoclonal antibodies, was extensively reviewed by Rosenberg and Terry (32), who concluded that the results were disappointing. Therapeutic benefit was largely limited to those situations in which immune serum is administered almost immediately after tumor challenge. The two exceptions are the studies of sarcomas induced by Moloney sarcoma virus (33) and of tumors induced by polyoma virus (34). The high immunogenicity of the Moloney sarcoma (as evidenced by its spontaneous regression when induced in adult mice) doubtless facilitated the immunotherapeutic success of the immune serum. The prolongation of survival of rats bearing polyoma virus-induced tumors required the administration of large quantities of immune serum (the equivalent of almost five times the rat's blood volume).

In reviewing human trials of serotherapy (prior to monoclonal antibodies), Rosenberg and Terry (32) could find no convincing evidence that immune serum from any source had had a beneficial effect on the clinical course of cancer. Positive reports on the use of allogeneic serum from cured patients, such as the report of Ngu (35), are not well-documented and have not been corroborated (36). The successes claimed with the use of immune heterologous sera are poorly detailed and have not been confirmed by others (37,38). The possible reasons for failure are many and include inappropriate specificity, low avidity, ineffective isotype, and limited availability of high-titer sera.

The hybridoma technology developed by Kohler and Milstein (39) has enabled production of large quantities of homogeneous antibody of defined specificity, isotype, and function. Foon et al (40) have reviewed the animal tumor model studies with these reagents. The studies of Bernstein and co-workers (41-43) nicely illustrate the therapeutic promise and define the potential limitations of monoclonal antibodies. Employing subcutaneous implants of the SL2 leukemia in AKR mice and murine monoclonal antibodies directed against the Thy 1 differentiation antigen on the leukemic and normal T cells, they made the following observations. (*a*) Antibodies of the IgG2a isotype which are active in antibody-dependent cellular cytotoxicity (ADCC) were most effective. (*b*) Antibodies worked best in preventing hematogenous dissemination. (*c*) When hematogenous dissemination occurred, it was often associated with nonexpression of the Thy 1 antigen. (*d*) Subcutaneous tumor transplants were more resistant to therapy than blood-borne tumor cells. To achieve antigen saturation and inhibition of tumor growth, large quantities (3.2 mg) of monoclonal antibody were required. However, the use of extremely high doses led to suboptimal therapeutic effects. (*e*) The maximum subcutaneous implant which could be consistently eliminated was small (3×10^5 cells). This limitation was felt to be due to inadequate participation by host effector cells in clearing antibody-coated targets. Johnson et al (44) have shown that this restriction applies to individual sites and that antibody can prevent the growth of multiple implants of 10^5 cells in a single mouse.

Murine monoclonal antibodies directed against the common acute lymphoblastic leukemia antigen (CALLA) or a pan-T-cell antigen have been used to treat a number of patients with leukemia and lymphoma (40,45). The most consistent finding is a dramatic but transient fall in circulating leukemia or lymphoma cells, with recovery to pretreatment levels within 24 hours. Antigenic modulation occurs frequently, but the modulated antigens are re-expressed. In patients with cutaneous T-cell lymphoma, skin and lymph node deposits have regressed, but these regressions were of short duration and have not exceeded 4 months (46). The one published dramatic result was that of Miller and co-workers (47), who raised a monoclonal antibody to the idiotypic determinants on a patient's B-cell lymphoma. Treatment with this antibody resulted in a complete remission of > 1 year's duration.

The availability of large quantities of monoclonal antibody of known isotype and defined specificity seems to have improved the therapeutic efficacy of serotherapy in both animals and man. However, so far, clinically meaningful regressions are infrequent and there are significant impediments to further improvement. Antigenic modulation seems a frequent finding. This problem, as well as heterogeneity of antigen expression and seroselection of low antigenic variants, may perhaps be overcome by the use of a pool of antibodies. The apparent outcome-limiting capacity of host ADCC effector cells to eliminate antibody-coated targets is a formidable obstacle, even though recent data suggest that lymphokines (48) and interferon (49) can facilitate ADCC. In patients treated with murine monoclonal antibodies, the development of antibodies to the mouse immunoglobulin has been a problem. The use of human monoclonal antibodies could reduce the development of a host antimonoclonal antibody. However, in some cases, the immune response is to idiotypic determinants and might occur even with monoclonal antibody of completely human origin. It is probably unrealistic to anticipate that antibody alone can reliably effect clinically meaningful tumor regressions.

Theoretically, the treatment potential of native monoclonal antibodies can be enhanced by using these molecules as carriers for toxins, chemotherapeutic agents, and therapeutic isotopes. As the potency of these reagents is improved, it becomes increasingly likely that normal tissue damage will become dose-limiting. Particular attention must be paid to the suggestion by Brown (50) that monoclonal antibodies, while molecularly homogeneous, may be multispecific, exhibiting several different binding capabilities, and that use of these antibodies in a complex antigenic environment increases the likelihood that unwanted binding activities involving related (cross-reactive) or unrelated ("linked") determinants will be observed. Further, the reticuloendothelial system will clear aggregated or immune complexed immunoglobulin and large amounts of antibody may concentrate in the kidneys. Key et al (51) have demonstrated murine monoclonal antibodies in the renal tubule cells of treated guinea pigs bearing the line 10 tumor. The damage done to normal tissues during the exercise of these physiologic functions will depend on the potency of the effector molecules coupled to the carrier antibody. These are not trivial issues, but are rather formidable obstacles to the successful systemic treatment of human tumors with monoclonal antibody conjugates. Early enthusiasm must be moderated by this reality.

Immunotherapy With Cells

Early studies of adoptive therapy of established tumors with immune cells met with some success. Several workers induced regression of both autochthonous and transplanted tumors in animals by the administration of immunized cells (52-54). In some studies, allogeneic or heterologous cells were effective, although not as effective as syngeneic cells. A common requirement for successful therapy was the use of large numbers of lymphocytes. Early attempts to utilize cell transfer immunotherapy in humans were hampered not only by the need for a large number of cells, but also by the unavailability of suitable immune cells. The cross-immunization of cancer patients with subsequent transfer of immune cells was best studied by Nadler and Moore (55,56), who reported that immune cell transfer was capable of affecting the growth of tumors in humans. Of 118 patients with advanced cancer, 23 had objective responses. However, documentation of many responses was not presented. These studies and the results of similar attempts by Humphrey et al (57) implied that at least small therapeutic effects are achievable with adoptive immunotherapy in some patients with advanced cancer.

Interest in adoptive cellular immunotherapy has been rekindled by two developments: the appreciation that the abrogation of host T-cell suppressor function can facilitate adoptive immunotherapy and the discovery that cytotoxic T cells could be expanded in vitro in the presence of Interleukin 2 (IL-2). Working with the murine Meth A fibrosarcoma, Berendt and North (58) were able to induce complete regression of large established tumors by iv infusion of sensitized T cells from immune donors. However, success was achieved only when the tumors were growing in thymectomized T-cell-deficient recipients. The inhibition of this therapeutic effect by the infusion of T cells from immunologically intact tumor-bearing hosts that had lost concomitant immunity indicates a modulatory role for suppressor T cells. Subsequently, North (59) demonstrated that a cyclophosphamide-treated, tumor-bearing recipient mouse can substitute for a thymectomized and irradiated recipient for demonstrating complete regression of an established tumor by adoptive transfer of tumor-sensitized T cells. Mills and North (60) concluded that suppressor T cells inhibit adoptive immunotherapy by inhibiting the production of cytolytic T cells in the tumor-bearing recipient.

A continuing impediment to clinical trials is the need for significant quantities of tumor-immune autologous lymphocytes. The discovery (61) and subsequent purification (62) of human T-cell growth factor (IL-2) seem to have provided a means for circumventing this obstacle. Adding IL-2 to the medium allows the long-term culture of activated T cells. Cells expand at a rapid rate and it is technically possible to generate kilograms of lymphoid cells in a few months. Eberlein et al (63) have cured palpable local and disseminated FBL-3 tumor growing in irradiated C57BL/6 mice with a single transfer of in vivo immunized, in vitro sensitized cells expanded in vitro > 3000-fold in IL-2. Cheever et al (64) have also successfully treated an established FBL-3 tumor by infusion of in vivo immunized, in vitro sensitized, and IL-2-cultured lymphocytes preceded by a noncurative dose of cyclophosphamide.

In attempting to adapt this technology to human use, Rosenberg et al (65) exposed peripheral blood lymphocytes from cancer-bearing patients to IL-2 in vitro and were able to demonstrate cytotoxicity to autologous fresh tumor. Limiting dilution techniques were used to isolate individual clones with autologous antitumor reactivity and these clones were successfully expanded 10,000-fold in IL-2. Mokyr and Dray (66) have recently reviewed procedures for in vitro immunization. IL-2 can also be used to expand lymphocyte clones with natural killer activity (67). Further in vitro exposure of peripheral blood lymphocytes to IL-2 induces a lymphokine-activated killer (68) function which is effective against natural killer cell-resistant targets. Both cell types have the potential for use in adoptive immunotherapy.

In summary, the preponderance of data accumulated over the past two decades supports the notion that cellular mechanisms are more important than native antibody in inhibiting tumor growth. Recent developments which allow circumvention of the T-cell suppressor mechanism and propagation of large numbers of autologous tumor-specific lymphocytes in vitro have justifiably rekindled interest in adoptive immunotherapy. The therapeutic potential of native monoclonal antibodies seems to be less than that for immune cells, and the efficacy of monoclonal antibody conjugates may well be limited by toxicity to normal tissues

Does Tumor Burden Represent a Critical Limitation to Immunotherapy?

Clinical therapeutic immunology has had its greatest impact in the prevention of infectious diseases (69). The study of the efficacy of immune therapy for established infectious diseases was abruptly curtailed by the development of antibiotics. Nonetheless, some success was achieved with the immunotherapy of microbial disease, for example, with antipneumococcus rabbit serum as a therapeutic agent of lobar pneumonia in man (70). Will antitumor immunization only be effective as immunoprophylaxis, or can immunotherapy be effective against clinically relevant tumor burdens? In an effort to address this question, we will look at what has already been accomplished in the immunotherapy of tumor-bearing animals and man.

It has been difficult to demonstrate a therapeutic benefit for active specific immunization in tumor-bearing animals (71–74) or man (4,75,76). It would be unwise to assume that the reasons for failure were the same for both groups. However, a commonly proffered explanation is that specific immunity can only cope with minimal tumor burdens. In almost all animal tumor models, there is an inverse relationship between tumor burden and therapeutic outcome. Nowhere is this more apparent than in the multitude of immunoprophylaxis studies. There is almost always a challenge dose that will overwhelm the host's immune system. In "tumor-bearing" models, therapy is often started the day after tumor transplant. With each day's delay, the therapeutic outcome diminishes. Finally, the observation of concomitant immunity, wherein an animal with a progressively growing "large" tumor can successfully reject a small tumor challenge (77), strongly implies that the immune system can only cope with tumor deposits of minimal size.

These studies tend to define a "small tumor burden" in a mouse as $\leqslant 10^6$ cells. If the immune system of a 20-g mouse can reject 10^6 tumor cells, what is the tumor cell-rejecting capacity of a 70-kg man? If we use ratio of body weights as a multiplication factor, then 1 × 10^6 tumor cells in a mouse is the biologic equivalent of 3500 × 10^6 or 35 × 10^8 tumor cells in a 70-kg man (roughly a spherical mass of tumor of about 2.5 cm in diameter). If we use the more conservative ratio of body surface areas as a multiplication factor, then the corresponding number is about 2.6 × 10^8 tumor cells (roughly a 0.5-cm diameter mass). By either method of calculation, the human system should be capable of rejecting a small, but clinically evident, tumor. However, recently it has been shown in several different tumors that, under the proper circumstances, a mouse can immunologically reject a much larger tumor. Berendt and North (58) demonstrated immunologic rejection of 5-mm diameter tumors in suppressor cell-depleted mice. Hengst et al (78,79) and Mokyr et al (80), working with MOPC-315 tumor-bearing mice, demonstrated that a single ip injection of cyclophosphamide (low dose, 15 mg/kg) cured most mice bearing large 25-mm (Day 14) tumors, whereas injection into mice bearing nonpalpable (Day 4) tumors cured only a few of the mice. This group has provided strong evidence that the treatment of the large Day-14 tumors was successful because adequate time had elapsed to allow development of an antitumor immune response which was expressed when the cyclophosphamide abrogated suppressor cell function. Using either of the calculations described above, a 25-mm tumor in a 20-g mouse corresponds to a massive tumor in a 70-kg man.

In man, there is more circumstantial evidence which casts doubt on the dogma that the immune response can cope with only minimal tumor burdens. The immune response can cause regression of rather large organ transplants. However, critics would argue, and perhaps rightly so, that this is accomplished on the basis of a vasculitis. Nonetheless, regressions have been noted of accidentally transplanted tumors when immunosuppressive measures are stopped (81). Although the mechanisms of spontaneous tumor regressions are unknown, an immunologic component is suggested. With malignant melanoma, there are reports of spontaneous regression of rather large visceral metastases (82,83). We have observed spontaneous regression of multiple pulmonary metastases, each measuring > 1 cm in diameter in one patient, and multiple subcutaneous metastases of similar size in another patient.

Taken as a whole, these observations suggest that, given the proper circumstances of suppressor T-cell depletion, tumor-specific immunity can cope with much larger tumor burdens than is generally appreciated. This contention is readily subject to testing in animals bearing established immunogenic tumors, by immunizing these animals following the administration of cyclophosphamide. Unfortunately, such studies have not been reported. One approach to testing this concept in humans is to administer tumor vaccines following depletion of suppressor cell function. Our studies (84) of skin test reactivity to autologous tumor following cyclophosphamide and tumor immunization are not designed to address the issue of tumor regression. However, it is encouraging that the first patient so treated has had a > 50% regression of numerous dermal metastases and a complete regression of a pulmonary nodule.

It is reasonable to conclude that with the abrogation of suppressor mechanisms and the availability of large quantities of specifically immune serum and cells, clinically significant tumor burdens can be made to regress.

Is Nonspecific Cytotoxicity Relevant to the Rejection of Tumors?

Macrophages

Macrophages can effect specific cytolysis as the cellular mediators of ADCC, where immunologic specificity is provided by antibody. Macrophages also serve as antigen-presenting cells and, in this capacity, facilitate the acquisition of T-cell-mediated and humoral immunity. However, here we are concerned with the unique characteristic of macrophages to be rendered nonspecifically cytotoxic (static or lytic) to tumor cells in vitro—a phenomenon commonly referred to as activation (85,86). Macrophages are activated by lymphokines released consequent to a tumor-specific or other immune response (87). They can also be activated by a variety of other stimuli including endotoxin (88), synthetic polymers (89), interferon (90), and micro-organisms such as BCG (91) and *Corynebacterium parvum* (92). The latter two agents have been extensively tested as treatments for human cancer (92-94).

The in vitro cytotoxicity of activated macrophages was first demonstrated in growth inhibition or "cytostasis" assays (95). Subsequently, it was shown that activated macrophages could also be cytolytic, ie, kill tumor cells directly (96). In vitro macrophage-mediated, nonspecific cytotoxicity is of great interest to cancer immunotherapists, because it is not dependent on the development of an immune response against tumor-specific antigens. However, the in vivo significance of this type of cytotoxicity is open to question.

Macrophages are a central component of the reticuloendothelial system and are widely distributed in the spleen, lymph nodes, liver, pulmonary alveoli, serous cavities, and connective tissue. They are often found infiltrating tumors. However, the in vivo role of macrophages in resistance to tumor growth is difficult to assess. The primary approach to studying this problem is to examine the effects of macrophage activators on tumor growth. As cited above, macrophages can be activated by a variety of substances. The two most commonly used are BCG and *C. parvum*. However, in vivo, these agents can also serve as adjuvants and can facilitate the acquisition of T-cell-mediated and humoral immunity. When used in conjunction with immunogenic tumors, it is often difficult to distinguish specific T-cell-mediated cytotoxicity from nonspecific macrophage-mediated cytotoxicity. For example, it is true that in immunoprophylaxis experiments, the administration of BCG and *C. parvum* can protect experimental animals from transplantation of tumors (92,93). However, much of this work has been performed with strongly immunogenic tumors, so that a specific T-cell-mediated, rather than a nonspecific macrophage-mediated, mechanism could be involved. In fact, Smith and Scott (97) and Hewitt and Blake (98) reported that *C. parvum* was ineffective in protecting animals from nonimmunogenic tumors. A few investigators (89,99) have demonstrated the effectiveness of macrophage-activating agents against tumors that had been shown to be nonimmunogenic by the conventional immunization-rechallenge method. However, it is difficult to exclude weak immunogenicity, such as that conferred by contaminating viral antigens (100), which might become significant when an adjuvant is administered.

There are reports that adoptively transferred activated macrophages can confer protection that is nonspecific and does not require the participation of the recipient's lymphocytes (99), but there are also observations to the contrary (101,102).

To date, attempts at systemic macrophage activation as therapy of established experimental tumors have shown little effectiveness. The large number of studies performed with BCG and *C. parvum* have been reviewed rather extensively (91-93). In general, the therapeutic effect has been modest; that is, there has been retardation of tumor growth, rather than tumor regression and cure. Further, success has been achieved under such strictly defined conditions that duplication of results in other laboratories is often difficult and extension to other species is almost impossible. The frequency of this inconsistency may result from the facts that the models in studies reported in the literature are likely carefully chosen from a larger number of negative systems and thus represent only a small fraction of the models tested; that the therapeutic effects are meager; and that test conditions are critical. Attempts to extrapolate these models to the clinic, therefore, would be hopeless.

Similarly, in humans, the "systemic" use of macrophage-activating agents to treat established tumors has been generally minimally effective. There are a few exceptions. Moertel et al (103) reported three objective responses in 26 patients with advanced gastrointestinal cancer treated with methanol-extracted residue of BCG (MER). Three of seven patients with melanoma experienced objective remissions when treated with oral BCG (104). Objective responses were noted in eight of 20 patients with a variety of advanced cancers treated with iv *C. parvum* (105). However, none of these studies has had independent confirmation. Further, there are many more studies in which no antitumor effect has been noted (94).

The lack of therapeutic success with the systemic administration of macrophage stimulators in tumor-bearing animals and in man may result from an inability of currently available agents physically to reach tumor-resident macrophages, a failure of agents like BCG and *C. parvum* to activate relevant macrophages to maximum cytotoxicity, or an inherent inability of maximally activated macrophages to mediate substantive tumor regression. Access to tumor-resident macrophages may be a relevant issue, since the most striking therapeutic successes with BCG and *C. parvum* have been with their local or intralesional use. The best example of this phenomenon is the guinea pig hepatoma model. Working with an inbred strain of guinea pigs and a transplantable hepatocarcinoma, Zbar et al (106) demonstrated that intralesional BCG can induce regression of established dermal tumor transplants, as well as stimulate tumor-specific cell-mediated immunity. They found that tumor regression required an intact immune system and direct contact between BCG and tumor cells. The therapeutic efficacy of intralesional therapy has been extended to a spontaneous primary ocular cancer in cattle (107).

Similarly striking and equally reproducible results have been achieved in man in the treatment of intradermal melanoma metastases. Regression of injected as well as uninjected lesions has been well-documented (108). This work has been extended to the presurgical treatment of primary cutaneous melanomas. Rosenberg et al (109) randomized 26 high-risk stage I patients to conventional surgical therapy (wide excision plus regional lymph node dissection) with or without preoperative intralesional BCG. With a median follow-up of 3.3 years, significantly fewer recurrences were seen in the BCG-treated group (five of 13 patients) than in the controls (ten of 13). These preliminary results will require confirmation. It also appears that intravesical BCG, when combined with conventional therapy, is useful in the management of recurrent superficial bladder cancer (110-113). A positive therapeutic effect is consistently reported and is characterized by a prolongation of time to recurrence and a decrease in the number of recurrences. When compared directly with intravesical thiotepa (113), BCG was more effective in preventing tumor recurrence.

There is considerable controversy over whether activated macrophages are primarily responsible for the antitumor effects of local BCG or *C. parvum* therapy in animals and in man. *C. parvum* and BCG are immunoadjuvants that augment cell-mediated and humoral immune responses when injected at the same site as antigen. Success in the guinea pig hepatoma model requires an intact immune system. However, this requirement may relate only to the need to generate macrophage-activating lymphokines via a host anti-BCG response. Further, the development of tumor-specific transplantation immunity may be the result, rather than the cause, of tumor regression. North et al (114) attempted to resolve this issue.

Working with the P815 mastocytoma in syngeneic DBA/2 mice, they demonstrated that regression of tumor admixed with *C. parvum* required an intact immune system; local tumor regression failed to occur in T-cell-deficient mice, suggesting that nonspecific macrophage-mediated cytotoxicity did not play a major role in tumor regression. Further, regression of uninjected tumor deposits was temporally associated with the generation of T-cell-mediated tumor-specific immunity and an immunologically unrelated tumor did not regress. Regressive capacity decayed as T-suppressor cell function developed. Thus, in this model, tumor-specific T-cell-mediated immunity was responsible for tumor control. In man, during the course of intralesional BCG therapy of dermal melanoma metastases, regression of uninjected lesions is seen most often when dermal metastases are confined to an extremity and the uninjected lesions are in close proximity to sites of BCG injection. At present, in man, there are not reliable in vitro methods with which to quantitate tumor-specific T-cell-mediated antitumor immunity or tissue macrophage activation. Occasionally, regression of visceral metastases is noted (115), suggesting a systemic effect beyond the field of regional macrophage activation. Unfortunately, suitable experiments cannot be performed in man with current techniques to resolve this issue.

In summary, a decade has elapsed since the original description of in vitro macrophage-mediated antigen-independent tumor cell cytotoxicity. Precise definition of a substantive in vivo role for this phenomenon in the inhibition of tumor growth remains elusive. This results largely from the fact that the commonly employed macrophage activators are also immunologic adjuvants which facilitate the acquisition of tumor-specific immunity. Experiments with nonimmunogenic tumors and adoptive transfer studies could help clarify this issue, but unfortunately, the results of these studies are conflicting. Even with intralesional and local therapy, where antitumor effects have been most striking, an exclusive role for nonspecifically activated macrophages is not certain. There is a continuous search for more effective local and systemic macrophage activators and such agents may be useful in the generation and amplification of tumor-specific immunity. However, even if the theoretic problems can be overcome, the selective targeting of systemically administered agents to activate tumor-resident macrophages remains a formidable task.

Natural Killer (NK) Cells

A second type of cell that seems to be cytotoxic to tumors without a requirement for tumor-specific antigens is the natural killer (NK) cell. NK cells appear in normal individuals (man and mouse) without known prior immunization. The history and significance of these cells have recently been reviewed (116). NK cells are large granular lymphocytes which express Fc receptors and form low-affinity rosettes with sheep red blood cells. In vitro, NK cells are spontaneously cytolytic for tumors and some nonmalignant target cells. This cytotoxicity occurs in the absence of primary immunization, exhibits no immunologic memory, and is not major histocompatibility-complex-restricted. A considerable research effort has focused on the definition of an in vivo role in the regulation of tumor growth for this in vitro phenomenon. Nude (T-cell-deficient) mice do not have a high incidence of spontaneous or carcinogen-induced tumors (117). This has led to speculation on the existence of a natural cellular immune system consisting of NK cells, macrophages, and polymorphonuclear neutrophils that accomplishes surveillance against tumors (118). Tumor growth is facilitated in nude mice in which NK-cell function is impaired by age (119) or antibody (120). These data suggest an in vivo role for NK cells in modulating tumor growth and metastasis. However, as discussed by Roder and Pross (121), this hypothesis must take into account several seemingly paradoxical observations to include relatively normal NK activity in many cancer patients with early-stage disease, the relative insensitivity of fresh human tumor cells to in vitro lysis by NK cells, and the often low level of NK activity of tumor-infiltrating cells. One explanation for these observations is that tumor progression is associated with the acquisition of resistance to lysis by NK cells. However, at least one animal model casts doubt on the general applicability of this hypothesis. Urban et al (122) have demonstrated that progressor variants of spontaneously regressing ultraviolet radiation-induced fibrosarcomas lose their in vitro sensitivity to lysis by tumor-specific T cells, but retain sensitivity to lysis by NK cells. Thus, the precise role of NK cells in modulating in vivo tumor growth is uncertain.

In summary, the available data raise serious questions about whether nonspecific killing of tumor cells by NK cells is an important component of tumor rejection. Thus, agents that act primarily by stimulatig NK cells may not be therapeutically promising. However, macrophages are essential elements in the generation and amplification of tumor-specific immune responses and agents that stimulate them might be useful as adjuncts to specific immunotherapy.

Is the Presence of Tumor-Specific Suppressor Cells a Serious Impediment to Successful Immunotherapy?

As documented above, great difficulty has been encountered by investigators attempting to demonstrate that patients develop an immune response against tumor-associated antigens. One explanation for this difficulty is that tumor antigens are not recognized by the autologous host as foreign and that an immune response is never initiated. However, an alternative explanation is that tumor cells are immunogenic, but that the host in which they arise becomes tolerant to them.

Although the mechanisms of immunologic tolerance are incompletely understood, evidence from many experimental systems suggests that antigen-specific suppressor T lymphocytes play a major role (123,124). Tumor-associated antigens may induce immunologic tolerance, and the failure of the host to make an effective immune response could be due to the activity of specific suppressor cells.

The reasons for considering such a hypothesis have been reviewed (125,126). There are several experimental systems in which tumor-specific suppressor cells appear shortly after the transplantation of syngeneic tumors: (*a*) the methylcholanthrene-induced sarcoma system of Fujimoto et al (127); (*b*) the P815Y mastocytoma system of Takei et al (128); (*c*) the ultraviolet-induced sarcoma system of Kripke (129); and (*d*) the Meth A sarcoma model of North et al (114). In these systems, it appears that in the initial stages of growth, tumors stimulate a specific measurable immune response, but that this response is transient because of the development of T cells that suppress it.

Patients with primary cancers, even those detected "early," probably correspond immunologically to mice with well-established tumors, and it is reasonable to hypothesize that the tumor-directed immune response of these patients has already passed through the initial phase and is in a suppressor phase at the time of diagnosis. This hypothesis would account for the difficulty in measuring tumor-directed immunity and also for the failure of conventional forms of immunotherapy. The suppressor hypothesis predicts that a reduction in tumor-specific T-suppressor activity would facilitate the successful use of immunotherapy.

There are multiple approaches to reducing T-cell suppressor function. Cyclophosphamide is the approach being most intensively studied at this time (130). Interest in cyclophosphamide as an immunopotentiator begins with the work of Maguire and Ettore (131). They discovered that guinea pigs treated with cyclophosphamide and then contact-sensitized to DNCB developed much more intense and prolonged allergic contact dermatitis reactions than did control guinea pigs that had received sensitizer alone. In addition, cyclophosphamide has been shown to immunopotentiate the acquisition of delayed-type hypersensitivity to a variety of microbial products and to ovalbumin (132–134). Antibody responses can also be heightened by cyclophosphamide (135). These and other studies clearly demonstrate that cyclophosphamide can potentiate the development of cellular and humoral immunity to new antigens in animals. Of equal importance is the ability of cyclophosphamide to breach natural and acquired tolerance. Yoshida et al (136) were able to induce delayed-type hypersensitivity (foot pad swelling) in mice to syngeneic or autologous testicular cells by immunization with these tissues, only if preceded by cyclophosphamide. Polak and Turk (137) showed that unresponsiveness to the dinitrophenyl group in guinea pigs could be broken by treatment with cyclophosphamide just before sensitization; this was associated with the return of the ability of T cells to proliferate in the draining lymph node in response to sensitizer.

North et al (114) and Berendt and North (58) have described a system in which adoptive transfer of tumor-immune spleen cells to immunologically intact tumor-bearing mice had no effect on tumor growth. However, if mice had been depleted of T cells by thymectomy, lethal irradiation, and bone marrow reconstitution, or by pretreatment with cyclophosphamide, then adoptive transfer of immune spleen cells resulted in complete and permanent regression of established tumors. The tumor regression could be prevented by administration of splenic T cells from normal mice in which that tumor was growing progressively, but not by spleen cells from normal mice. Thus, depletion or impairment of cyclophosphamide-sensitive suppressor T cells allowed the expression of immunity to tumor-associated antigens.

There are a number of other studies in rodent models suggesting that the principle of cyclophosphamide immunopotentiation might be applied to heighten the immune response to tumor antigens. Cyclophosphamide pretreatment followed by tumor cells increased the number of cytotoxic T cells, as judged by in vitro cytotoxicity or by Winn testing (138). Low-dose cyclophosphamide treatment of MOPC-315 myeloma in syngeneic mice decreased tumor growth by immunologic means (78). Cyclophosphamide treatment of mice bearing an MCA fibrosarcoma resulted in the maintenance or restoration of tumor immunity, as judged by the activity of spleen cells in a Winn assay (139).

Curiously, the particularly pertinent issue of using cyclophosphamide to immunopotentiate a tumor vaccine to cause rejection of an established syngeneic tumor has not been investigated in an animal model. The ability of cyclophosphamide immunopotentiation to facilitate the immunologic rejection of autologous tissue and its ability to break natural and acquired specific immunologic tolerance when used with antigen (136,137) provide precedent and encouragement that a similar immunologically based rejection of autologous (syngeneic) tumor tissue can be obtained.

Stimulated by these observations, Berd and co-workers (140) studied the effect of cyclophosphamide on acquisition of delayed-type hypersensitivity in tumor-bearing patients. They demonstrated an enhanced acquisition of delayed-type hypersensitivity to the neoantigen, KLH. All 11 patients developed a positive response to KLH (median response diameter, 19 mm) when sensitized following administration of cyclophosphamide, whereas only four of 11 patients developed positive responses (median response diameter, 0 mm) when immunized without cyclophosphamide. These studies were extended to skin testing with autologous tumor cells (84). Enhancement of skin test reactivity to autologous tumor has been demonstrated in six of six patients tested to date. In no case was enhancement of reactivity to normal control tissues demonstrated.

The studies cited above deal with the phenomenon of potentiation of the acquisition of immunity by cyclophosphamide, rather than the mechanism of this effect. Currently, the leading hypothesis for explaining this immunopotentiation is that cyclophosphamide works by selectively depleting suppressor T cells (by depletion of the pool of presuppressors prior to immunization). Ozer et al (141) showed that in vitro treatment with 4 hydroperoxycyclophosphamide, an active metabolite of cyclophosphamide, completely inhibits the development of concanavalin A-inducible suppressor T cells.

In summary, the realization that apparently tumor-specific suppressor cells develop in response to growing malignant tumors has opened an important new area of research in tumor immunology.

CONCLUSIONS

These are difficult times for tumor immunology and those who study it. The failure of most attempts to apply experimental immunotherapy data to the treatment of human cancer has generated much skepticism. Yet, it is reasonable to argue that in most cases, the extrapolations from mouse experiments to human studies have been inappropriate and that clinical studies with a clear and firm experimental basis have met with some success.

For example, the systemic administration of nonspecific immunostimulants, such as BCG, is effective in preventing the growth of experimental tumors, ie, when BCG is administered days to weeks before the tumor is implanted. Incorrect extrapolation of this observation to clinical trials in which BCG was given to patients with established metastatic cancer, albeit often micrometastatic cancer, resulted in failure. On the other hand, the injection of BCG directly into a tumor often results in regression of established experimental tumors and, likewise, has been found to be effective in human cancer, eg, dermal metastases of malignant melanoma.

It is important that we avoid these types of incorrect extrapolations in future clinical trials. Tumor-specific antibody, when used alone, has not been a substantive treatment of transplanted animal tumors, and it is not likely to be any more effective in human cancers. The role of nonspecific cytotoxic mechanisms in modulating tumor growth in vivo is uncertain. Until this core issue is resolved, agents that stimulate nonspecific cytotoxic mechanisms do not have much promise for the treatment of human malignancies, except in very special circumstances. Of course, such agents might be of value as immunologic adjuvants. Finally, active specific immunotherapy, ie, the use of vaccines in patients with established tumors, even micrometastatic tumors, is almost certain to fail, unless accompanied by a drug or biologic that can reverse what appears to be immunologic tolerance to the tumor cells. Reduction of suppressor cell activity by cyclophosphamide is one approach for reversing immunologic tolerance, but more selective methods may be discovered as we attain a greater understanding of how immune responses are regulated.

We are still not certain whether human tumors bear "unique," anatomically specific, or "unusual," functionally specific antigens. The transplantation experiments performed in rodents obviously cannot be reproduced in human subjects. Monoclonal antibody studies have been inconclusive. In vitro techniques, such as lymphocyte-mediated cytotoxicity and lymphoproliferative response to tumor antigens, have proven too crude to be helpful.

However, the evidence obtained from a large number of animal experiments and from a much smaller number of human studies suggests that there is something about a malignant tumor that renders it susceptible to immunologic rejection—under the proper circumstances. Research in tumor immunology over the next 10 or 20 years should have as its goals the definition and understanding of those proper circumstances.

REFERENCES

1. COLEY WB. Treatment of malignant tumors by repeated inoculations of erysipelas with a report of 10 cases. Med Rec 43:60-61, 1983.
2. GROSS L. Intradermal immunization of C3H mice against a sarcoma that originated in an animal of the same line. Cancer Res 2:327-333, 1943.
3. PREHN RT, and MAIN J. Immunity to methylcholanthrene induced sarcomas. JNCI 18:769-778, 1957.
4. MATHE G, AMIEL JL, SCHWARZENBERG L, ET AL. Active immunotherapy for acute lymphoblastic leukemia. Lancet 1:697-699, 1969.
5. MORTON DL. Immunological studies with human neoplasms. J Reticuloendothel Soc 10:137-160, 1971.
6. KLEIN E, HOLTERMANN OA, PAPERMASTER B, ET AL. Immunologic approaches to various types of cancer with the use of BCG and purified protein derivatives. Natl Cancer Inst Monogr 39:229-239, 1973.
7. BURMESTER BR. Immunity to visceral lymphomatosis in chickens following injection of virus into dams. Proc Soc Exp Biol Med 88:153-155, 1955.
8. FRIEND C. Immunological relationship among some of the murine leukemia viruses. *In* Viruses Inducing Cancer. Salt Lake City, University of Utah Press, 1966, pp 51-60.
9. LARSON VM, RAUPP WG, and HILLEMAN MR. Prevention of SV40 virus tumorigenesis in newborn hamsters by maternal immunization. Proc Soc Exp Biol Med 126:674-677, 1967.
10. BIGGS PM, PAYNE LN, MILNE BS, ET AL. Field trials with attenuated cell-associated vaccine for Marek's disease. Vet Rec 87:704-709, 1970.
11. HEWITT HB. Animal tumor models and their relevance to human tumor immunology. J Biol Resp Modif 1:107-119, 1982.
12. PREHN RT. Relation of tumor immunogenicity to concentration of the oncogen. JNCI 55:189-190, 1975.
13. SOUTHAM CM, and BEUNSCHWIG A. Quantitative studies of autotransplantation of human cancer. Cancer 14:971-978, 1961.

14. CLARK WH, MASTRANGELO MJ, AINSWORTH AM, ET AL. Current concepts of the biology of human cutaneous malignant melanoma. Adv Cancer Res 24:267-338, 1977.
15. OETTGEN HF. Host defense against cancer. *In* Cancer. New York, Grune & Stratton, Inc, 1981, pp 309-330.
16. MOLLER G. Immunological Reviews. Copenhagen, Munksgaard, 1980, vol 50, pp 227-273.
17. DIPPOLD WG, LLOYD KO, HOUGHTON AN, ET AL. Human melanoma antigens defined by monoclonal antibodies. *In* Hybridomas in Cancer Diagnosis and Treatment. New York, Raven Press, 1982, pp 173-182.
18. REISFELD RA, MORGAN AC, and BUMOL TF. Production and characterization of a monoclonal antibody to human melanoma associated antigen. *In* Hybridomas in Cancer Diagnosis and Treatment. New York, Raven Press, 1982, pp 183-186.
19. DE LEO AB, SHIKU H, TAKAHASHI T, ET AL. Cell surface antigens on chemically induced sarcomas of the mouse. I. Murine leukemia virus related antigens and alloantigens on cultured fibroblasts and sarcoma cells: description of a unique antigen on BALB/c Meth A sarcoma. J Exp Med 146:720-734, 1977.
20. PARMIANI G, and PIEROTTI MA. Generation of TSTA diversity. Looking for testable hypothesis. Cancer Immunol Immunother 14:133-136, 1983.
21. BERD D, PARKER G, SHIELDS J, ET AL. Autologous mixed lymphocyte-tumor cell (AMLTC) response in primary choroidal melanoma. Proc Am Assoc Cancer Res and ASCO 22:322, 1981.
22. HEWITT HB, BLAKE ER, and WALDER AS. A critique of the evidence for active host defense against cancer, based on personal studies of 27 murine tumors of spontaneous origin. Br J Cancer 33:241-259, 1976.
23. HEWITT HB. The choice of animal tumors for experimental studies in cancer therapy. Adv Cancer Res 27:149-200, 1978.
24. RAPP HJ. Appropriateness of animal models for the immunotherapy of human cancer. Cancer Res 39:4285-4287, 1979.
25. HERBERMAN RB. Counterpoint: animal tumor models and their relevance to human tumor immunology. J Biol Resp Modif 2:39-46, 1983.
26. HEWITT HB. Secondpoint: animal tumor models and their relevance to human tumor immunology. J Biol Resp Modif 2:210-216, 1983.
27. HERBERMAN RB. Second counterpoint: animal tumor models and their relevance to human tumor immunology. J Biol Resp Modif 2:217-226, 1983.
28. MASTRANGELO MJ, BERD D, and BELLET RE. Limitations, obstacles and controversies in the optimal development of immunotherapy. *In* Immunotherapy of Human Cancer. New York, Raven Press, 1978, pp 375-394.
29. OLD LJ, and BOYSE EA. Immunology of experimental tumors. Ann Rev Med 15:167-186, 1964.
30. KLEIN G. Tumor antigens. Ann Rev Microbiol 20:223-252, 1966.
31. GORER PA, and AMOS DB. Passive immunity of mice against C57B1 leukosis EL-4 by means of isoimmune serum. Cancer Res 16:338-343, 1956.
32. ROSENBERG SA, and TERRY WD. Passive immunotherapy of cancer in animals and man. Adv Cancer Res 25:233-288, 1977.
33. HELLSTROM I, and HELLSTROM KE. Colony inhibition studies on blocking and non-blocking effects on cellular immunity to Moloney sarcomas. Int J Cancer 5:195-201, 1970.
34. BANSAL SC, and SJOGREN HO. "Unblocking" serum activity in vitro in the polyoma system may correlate with anti-tumor effects of antiserum in vivo. Nature 233:76-78, 1971.
35. NGU VA. Host defenses in Burkitt's tumor. Br Med J 1:345-347, 1967.
36. FASS L, HERBERMAN RB, ZIEGLER J, ET AL. Evaluation of the effects of remission plasma on untreated patients with Burkitt's lymphoma. JNCI 44:145-149, 1970.
37. MURRAY G. Experiments in immunity in cancer. Can Med Assoc J 79:249-259, 1958.
38. DE CARVALHO S. Preliminary experimentation with specific immunotherapy of neoplastic disease in man. I. Immediate effects of equine immune gamma globulin. Cancer 96:306-330, 1963.
39. KOHLER G, and MILSTEIN C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495-497, 1975.
40. FOON KA, BERNHARD MI, and OLDHAM RK. Monoclonal antibody therapy: assessment by animal tumor models. J Biol Resp Modif 1:277-304, 1982.
41. BERNSTEIN ID, TAM RM, and NOWINSKI RC. Mouse leukemia: therapy with monoclonal antibodies against thymus differentiation antigen. Science 207:68-71, 1979.
42. BERNSTEIN ID, and NOWINSKI RC. Monoclonal antibody treatment of transplanted and spontaneous murine leukemia. *In* Hybridomas in Cancer Diagnosis and Treatment. New York, Raven Press, 1982, pp 97-112.
43. BADGER CC, and BERNSTEIN ID. Therapy of murine leukemia with monoclonal antibody against a normal differentiation antigen. J Exp Med 157:828-842, 1983.
44. JOHNSON RJ, SICILIANO RF, and SHIN HS. Suppression of antibody-sensitized cells by macrophages: insufficient supply or activation of macrophages within large tumors. J Immunol 122:379-382, 1979.
45. RITZ J, and SCHLOSSMAN SF. Utilization of monoclonal antibodies in the treatment of leukemia and lymphoma. Blood 59:1-11, 1982.
46. MILLER RA, and LEVY R. Response of cutaneous T cell lymphomas to therapy with hybridoma monoclonal antibody. Lancet 2:226-230, 1981.
47. MILLER RA, MALONEY DG, WARNICKI ER, ET AL. Treatment of B cell lymphoma with monoclonal anti-idiotype antibody. N Engl J Med 306:517-522, 1982.
48. RALPH P, WILLIAMS N, MOORE MAS, ET AL. Induction of antibody-dependent and nonspecific tumor killing in human monocytic leukemia cells by nonlymphocyte factors and phorbol ester. Cell Immunol 71:215-223, 1982.
49. BORDEN EC, HOLLAND JF, DAO TL, ET AL. Leukocyte-derived interferon (alpha) in human breast carcinoma. The American Cancer Society phase II trial. Ann Intern Med 97:1-6, 1982.
50. BROWN NA. Prospects for human monoclonal antibodies: a critical review. Yale J Biol Med 55:297-303, 1982.
51. KEY ME, BERNHARD MI, HOYER LC, ET AL. Guinea pig line 10 hepatocarcinoma model for monoclonal antibody serotherapy: in vivo localization of a monoclonal antibody in normal and malignant tissues. J Immunol 130:1451-1457, 1983.
52. DELORME EJ, and ALEXANDER P. Treatment of primary fibrosarcomas in the rat with immune lymphocytes. Lancet 2:117-120, 1964.
53. FEFER A. Immunotherapy and chemotherapy of Moloney sarcoma virus-induced tumors in mice. Cancer Res 29:2177-2183, 1969.
54. BORBERG H, OETTGEN HF, CHOUDY K, ET AL. Inhibition of established transplants of chemically induced sarcomas in syngeneic mice by lymphocytes from immunized donors. Int J Cancer 10:539-547, 1972.
55. NADLER SH, and MOORE GE. Clinical immunologic study of malignant disease: response to tumor transplants and transfer of leukocytes. Ann Surg 164:482-490, 1966.
56. ______. Immunotherapy of malignant disease. Arch Surg 99:376-381, 1969.
57. HUMPHREY LJ, JEWELL WR, MURRAY DR, ET AL. Immunotherapy for the patient with cancer. Ann Surg 173:47-54, 1971.
58. BERENDT MJ, and NORTH RJ. T-cell mediated suppression of antitumor immunity. An explanation for progressive growth of an immunogenic tumor. J Exp Med 151:69-80, 1980.
59. NORTH RJ. Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J Exp Med 55:1063-1074, 1982.
60. MILLS CD, and NORTH RJ. Expression of passively transferred immunity against an established tumor depends on generation of cytolytic T cells in recipient. Inhibition by suppressor T cells. J Exp Med 157:1448-1460.

61. MORGAN DA, RUSCETTI FW, and GALLO RC. Selective in vitro growth of T-lymphocytes from normal human bone marrows. Science 193:1007-1008, 1976.
62. WELTE K, WANG CY, MERTELSMANN R, ET AL. Purification of human interleukin 2 to apparent homogeneity and its molecular heterogeneity. J Exp Med 156:454-464, 1982.
63. EBERLEIN TJ, ROSENSTEIN M, and ROSENBERG SA. Regression of a disseminated syngeneic solid tumor by systemic transfer of lymphoid cells expanded in interleukin 2. J Exp Med 156:385-397, 1982.
64. CHEEVER MA, GREENBERG PD, and FEFER A. Specific adoptive therapy of established leukemia with syngenic lymphocytes sequentially sensitized in vivo and in vitro and non-specifically expanded by culture with interleukin 2. J Immunol 126:1318-1322, 1981.
65. ROSENBERG SA, EBERLEIN TJ, GRIMM EA, ET AL. Development of longterm cell lines and lymphoid clones reactive against murine and human tumors: a new approach to the adoptive immunotherapy of cancer. Surgery 92:328-335, 1982.
66. MOKYR MB, and DRAY S. In vitro immunization as a method for generating cytotoxic cells potentially useful in adoptive immunotherapy. Methods Cancer Res 19:385-417, 1982.
67. FLUMENBERG K, WELTE K, MERTELSMANN R, ET AL. Interleukin-2 dependent natural killer (NK) cell lines from patients with primary T cell immunodeficiencies. J Immunol 130:2635-2643, 1983.
68. GRIMM EA, RAMSEY KM, MAZUMDER A, ET AL. Lymphokine-activated killer cell phenomenon. II. Precursor phenotype is serologically distinct from peripheral T lymphocytes, memory cytotoxic thymus-derived lymphocytes and natural killer cells. J Exp Med 157:884-897, 1983.
69. PARISH HG. A history of immunization. London, E & S Livingstone, Ltd, 1965.
70. HORSFALL FL, GOODNER K, MACLEOD CM, ET AL. Antipneumococcus rabbit serum as a therapeutic agent in lobar pneumonia. JAMA 108:1483-1490, 1937.
71. ALEXANDER P. Immunotherapy of cancer: experiments with primary tumors and syngeneic tumor grafts. Prog Exp Tumor Res 10:23-71, 1968.
72. MATHE G, POUILLART P, and LAPEYRAQUE F. Active immunotherapy of L1210 leukemia applied after the graft of tumor cells. Br J Cancer 23:814-824, 1969.
73. RIOS A, and SIMMONS RL. Comparative effects of mycobacterium bovis and neuraminidase-treated tumor cells on the growth of established methylcholanthrene-fibrosarcomas in syngeneic mice. Cancer Res 32:16-21, 1972.
74. HOOVER HC, PETERS LC, BRANDHORST JS, ET AL. Therapy of spontaneous metastases with an autologous tumor vaccine in a guinea pig model. J Surg Res 30:409-415, 1981.
75. HOLLAND JF. Comparison of chemotherapy to chemotherapy plus VCN-treated cells in acute myelogenous leukemia. *In* Immunotherapy of Cancer: Present Status of Trials in Man. New York, Raven Press, pp 347-353.
76. LAUCIUS JF, BODURTHA AJ, MASTRANGELO MJ, ET AL. A phase II study of autologous irradiated tumor cells plus BCG in patients with metastatic malignant melanoma. Cancer 40:2091-2093, 1977.
77. VAAGE J. Concomitant immunity and specific depression of immunity by residual or reinjected syngeneic tumor tissue. Cancer Res 31:1655-1662, 1971.
78. HENGST JCD, MOKYR MB, and DRAY S. Importance of timing in cyclophosphamide therapy of MOPC-315 tumor-bearing mice. Cancer Res 40:2135-2141, 1980.
79. ______. Cooperation between cyclophosphamide tumoricidal activity and host anti-tumor immunity in the cure of mice bearing large MOPC-315 tumors. Cancer Res 41:2163-2167, 1981.
80. MOKYR MB, HENGST JCD, and DRAY S. Role of antitumor immunity in cyclophosphamide-induced rejection of subcutaneous nonpalpable MOPC-315 tumors. Cancer Res 42:974-979, 1982.
81. WILSON RE, HAGER EB, HAMPERS CL, ET AL. Immunologic rejection of human cancer transplanted with a renal allograft. N Engl J Med 278:479-483, 1968.
82. BULKLEY GB, COHEN MH, BANKS PM, ET AL. Long-term spontaneous regression of malignant melanoma with visceral metastases. Cancer 36:485-494, 1975.
83. NATHANSON L. Spontaneous regression of malignant melanoma: a review of the literature on incidence, clinical features and possible mechanisms. Natl Cancer Inst Monogr 44:67-76, 1976.
84. BERD D, MAGUIRE H, and MASTRANGELO MJ. Augmentation of delayed-type hypersensitivity to tumor-associated antigens by treatment with autologous tumor cell vaccine preceding cyclophosphamide. Proc ASCO 2:56, 1983.
85. ALEXANDER P. The role of macrophages in tumor immunity. J Clin Path 27 (Suppl 7):77-82, 1974.
86. HIBBS JB. Macrophage non-immunological recognition: target cell factors related to contact inhibition. Science 180:868-870, 1973.
87. FIDLER IJ. Inhibition of pulmonary metastases by intravenous injection of specifically activated macrophages. Cancer Res 34:1074-1078, 1974.
88. ALEXANDER P, and EVANS R. Endotoxin and double stranded RNA render macrophages cytotoxic. Nature New Biol 232:76-78, 1971.
89. MORAHAN PS, and KAPLAN AM. Macrophage activation and antitumor activity of biologic and synthetic agents. Int J Cancer 117:82-89, 1976.
90. SCHULTZ RM, CHIRIGOS MA, and HEINE UI. Functional and morphologic characteristics of interferon-treated macrophages. Cell Immunol 35:84-91, 1978.
91. MITCHELL MS, and MURAHATA RI. Modulation of immunity by Bacillus Calmette-Guerin (BCG). Pharmacol Ther 4:329-353, 1979.
92. BERD D. Effects of Corynebacterium parvum on immunity. Pharmacol Ther 2:373-395, 1978.
93. LAUCIUS JF, BODURTHA AJ, MASTRANGELO MJ, ET AL. Bacillus Calmette-Guerin in the treatment of neoplastic disease. J Reticuloendothel Soc 16:347-373, 1974.
94. MASTRANGELO MJ, BERD D, and BELLET RE. Critical review of previously reported clinical trials of cancer immunotherapy with nonspecific immunostimulants. Ann NY Acad Sci 277:94-133, 1976.
95. KELLER R. Cytostatic elimination of syngeneic rat tumor cells in vitro by non-specifically activated macrophages. J Exp Med 138:625-644, 1973.
96. ______. Susceptibility of normal and transformed cell lines to cytostatic and cytocidal effects exerted by macrophages. JNCI 56:369-374, 1976.
97. SMITH SE, and SCOTT MT. Biological effects of Corynebacterium parvum. III. Amplification of resistance and impairment of active immunity to murine tumors. Br J Cancer 26:361-367, 1972.
98. HEWITT HB, and BLAKE ER. Failure of pre-operative *C. parvum* vaccine to modify secondary disease following excision of two nonimmunogenic murine carcinomas. Br J Cancer 38:219-223, 1978.
99. FREEDMAN VH, CALVELLI TA, SILAGI S, ET AL. Macrophages elicited with heat-killed Bacillus Calmette-Guerin protected C57B1/6J mice against a syngeneic melanoma. J Exp Med 152:657-673, 1980.
100. SVET-MOLDAVSKY GL, LIOZNER AL, MKHEIDZE DM, ET AL. Tumor-induced skin heterogenization. II. Virus causing the phenomenon. JNCI 45:475-484, 1970.
101. PETERS HJ, MCBRIDE WH, MASON KA, ET AL. In vivo transfer of anti-tumor activity by peritoneal exudate cells from mice treated with Corynebacterium parvum: reduced effect in irradiated recipients. JNCI 59:881-887, 1977.
102. PARR IB, WHEELER E, and ALEXANDER P. Selective mobilization of specifically cytotoxic T-lymphocytes at sites of inflammation in relation to BCG-induced resistance to implants of syngeneic sarcoma in mice. JNCI 59:1659-1666, 1977.
103. MOERTEL CG, RITTS RE, SCHUTT AJ, ET AL. Clinical studies of methanol extraction residue fraction of Bacillus Calmette-Guerin as an immunostimulant in patients with advanced cancer. Cancer Res 35:3075-3083, 1975.
104. FALK RE, MANN P, and LANGEN B. Cell mediated immunity to human tumors. Arch Surg 107:261-266, 1973.
105. ISRAEL L, EDELSTEIN R, DEPIERRE A, ET AL. Daily intravenous

infusions of Corynebacterium parvum in twenty patients with disseminated cancer: a preliminary report of clinical and biological findings. JNCI 55:29-33, 1975.

106. ZBAR B, BERNSTEIN ID, and RAPP HJ. Suppression of tumor growth at the site of injection with living Bacillus Calmette-Guerin. JNCI 46:831-839, 1971.
107. KLEIN WR, RUITENBERG EJ, STEERENBERG PA, ET AL. Immunotherapy by intralesional injection of BCG cell walls or live BCG in bovine ocular squamous cell carcinoma: a preliminary report. JNCI 69:1095-1103, 1982.
108. MASTRANGELO MJ, and BERD D. Immunotherapy with microbial products. *In* Immunological Approaches to Cancer Therapeutics. New York, John Wiley & Sons, 1982, pp 75-106.
109. ROSENBERG SA, RAPP H, TERRY W, ET AL. Intralesional BCG therapy of patients with primary stage I melanoma. *In* Proceedings of the Second International Conference on the Immunotherapy of Cancer, Bethesda, MD, Apr 28-30, 1980.
110. MORALES A, EIDINGER D, and BRUCE AW. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol 116:180-183, 1976.
111. LAMON DL, THOR DE, STOGDILL VD, ET AL. Bladder cancer immunotherapy. J Urol 128:931-935, 1982.
112. CAMACHO F, PINSKY C, KERR D, ET AL. Treatment of superficial bladder cancer with intravesical BCG. Proc Am Assoc Cancer Res and ASCO 21:359, 1980.
113. BROSMAN SA. Experience with Bacillus Calmette-Guerin in patients with superficial bladder carcinoma. J Urol 128:27-30, 1982.
114. NORTH RJ, DYE ES, MILLS CD, ET AL. Modulation of antitumor immunity-immunologic approaches. Springer Semin Immunopathol 5:193-220, 1982.
115. MASTRANGELO MJ, BELLET RE, BERKELHAMMER J, ET AL. Regression of pulmonary metastatic disease associated with intralesional BCG therapy of dermal melanoma metastases. Cancer 36:1305-1308, 1975.
116. OLDHAM RK. Natural killer cells: history and significance. J Biol Resp Modif 1:217-231, 1982.
117. OUTZEN HC, CUSTER RP, EATON GJ, ET AL. Spontaneous and induced tumor incidence in germ free "nude" mice. J Reticuloendothel Soc 17:1-9, 1975.
118. HERBERMAN RB, and ORTALDO JR. Natural killer cells: their role in defenses against disease. Science 214:24-30, 1981.
119. MACHADO EA, LOZZIO BB, LOZZIO CB, ET AL. Study of metastases of human malignant cells in nude mice. *In* Proceedings of the Third International Workshop on Nude Mice. New York, Gustav Fischer, 1982, pp 391-402.
120. HANNA N, and BURTON RC. Definitive evidence that natural killer (NK) cells inhibit experimental tumor metastases in vivo. J Immunol 127:1754-1758, 1981.
121. RODER JC, and PROSS HF. The biology of the human natural killer cell. J Clin Immunol 2:249-262, 1982.
122. URBAN JL, BURTON RC, HOLLAND JM, ET AL. Mechanisms of syngeneic tumor rejection. Susceptibility of host-related progressor variants to various immunological effector cells. J Exp Med 155:557-573, 1982.
123. GERSHON RK, COHEN P, HENCIN R, ET AL. Suppressor T cells. J Immunol 108:586-589, 1972.
124. CANTOR H, and BOYSE E. Regulation of the immune response by T-cell subclasses. *In* Contemporary Topics in Immunobiology. New York, Plenum Press, 1977, pp 47-67.
125. BRODER S, and WALDMANN TA. The suppressor cell network in cancer. N Engl J Med 299:1281-1284, 1335-1341, 1978.
126. ROTH JA. Tumor induced immunosuppression. Surg Gynecol Obstet 156:233-240, 1983.
127. FUJIMOTO S, GREEN MI, and SEHON AH. Regulation of the immune response to tumor antigen. I. Immunosuppressor cells in tumor-bearing hosts. J Immunol 116:791-799, 1976.
128. TAKEI F, LEVY JG, and KILBURN DG. In vitro induction of cytotoxicity against syngeneic mastocytoma and its suppression by spleen and thymus cells from tumor-bearing mice. J Immunol 116:288-293, 1976.
129. KRIPKE ML. Immunological aspects of tumor induction by ultraviolet radiation. *In* Proceedings of the 13th International Cancer Congress, Seattle, WA, Sept 8-15, 1982, Abstract 3698.
130. BERD D, MAGUIRE HC, and MASTRANGELO MJ. Immunopotentiation by cyclophosphamide and other cytotoxic agents. *In* Immunomodulating Agents. New York, Marcel Dekker, 1983, in press.
131. MAGUIRE HC, and ETTORE VL. Enhancement of dinitrochlorobenzene (DNCB) contact sensitization by cyclophosphamide in guinea pigs. J Invest Derm 48:39-43, 1967.
132. KATZ SI, PARKER D, SOMMER G, ET AL. Suppressor cells in normal immunisation as a basic homeostatic phenomenon. Nature 248:612-614, 1974.
133. FINERTY JF, and KREHL EP. Cyclophosphamide pretreatment and protection against malaria. Infect Immun 14:1103-1105, 1976.
134. LIEW FY, RUSSELL SM, and BRAND CM. Induction and characterization of delayed-type hypersensitivity to influenza virus in mice. Eur J Immunol 9:783-790, 1979.
135. TURK JL, and PARKER D. Effect of cyclophosphamide on immunological control mechanisms. Immunol Rev 65:99-113, 1982.
136. YOSHIDA S, NOMOTO K, HIMENO K, ET AL. Immune response to syngeneic or autologous testicular cells in mice. I. Augmented delayed footpad reactions in cyclophosphamide-treated mice. Clin Exp Immunol 38:211-217, 1979.
137. POLAK L, and TURK JL. Reversal of immunological tolerance by cyclophosphamide through inhibition of suppressor cell activity. Nature 249:654-657, 1974.
138. GLASER M. Augmentation of specific immune response against a syngeneic SV40-induced sarcoma in mice by depletion of suppressor T cells with cyclophosphamide. Cell Immunol 48:339-345, 1979.
139. STEELE G, JR, SJOGREN HO, and ANKERS T. The effects of cyclophosphamide on in vitro correlates of immunity. Int J Cancer 14:743-752, 1974.
140. BERD D, MASTRANGELO MJ, ENGSTROM PF, ET AL. Augmentation of the human immune response by cyclophosphamide. Cancer Res 42:4862-4866, 1982.
141. OZER H, COWENS JW, COLVIN M, ET AL. In vitro effects of 4 hydroperoxycyclophosphamide on human immunoregulatory T subset function. I. Selective effects on lymphocyte function in T-B cell collaboration. J Exp Med 155:276-290, 1982.

Biologicals and Biological Response Modifiers: Fourth Modality of Cancer Treatment

Robert K. Oldham[1,*]

Considerable progress has been made in the last 5 years toward the development of specific biological approaches to the treatment of cancer. The techniques of genetic engineering, mass cell culture, and improved techniques in protein and nucleic acid sequencing and synthesis have made available biologicals as specific, highly purified molecules that can be used in cancer therapy. While extensive clinical trials are needed to demonstrate that biologicals and biological response modifiers can provide a survival advantage for patients with cancer, the preliminary data indicate that these approaches can be useful in inducing responses in patients with clinically apparent disease. Further studies with these approaches in conjunction with surgery, radiotherapy, and chemotherapy should be helpful in definitively establishing biologicals and biological response modifiers as the fourth modality of cancer treatment. [Cancer Treat Rep 68:221–232, 1984]

The three traditional modalities used in cancer treatment are surgery, radiation therapy, and chemotherapy. Each of these modalities has developed from its own historical context. Surgery clearly has the longest history as an effective modality of cancer treatment. However, the recognition that carcinomas spread regionally and often systemically made evident the need for other modalities. The recognition that radiation treatment could destroy cancer cells brought this modality into the clinical arena and radiotherapy has proven to be a highly effective method for further local-regional control of cancer.

The modern era of cancer treatment began in the 1940s and 1950s with the recognition that most cancers were systemic problems. It was recognized that often lymphatic and blood-borne metastases occurred simultaneously with local growth and regional spread and the need for systemic treatment became apparent. The early success of alkylating agents in the treatment of lymphoma prompted a massive search for drugs which might have cytolytic or cytostatic effects on cancer cells. Over the last two decades, there has been widespread recognition that drugs not only can effectively palliate in the treatment of cancer but also can cure. The development of these three modalities and their subsequent integration into what is now multimodal cancer treatment have been recently summarized (1).

In the last 5 years, we have reached another plateau in cancer treatment. New surgical techniques have been developed both for resectable tumors and for use in combined-modality treatment to allow radiotherapy and chemotherapy to be more effective (interoperative radiotherapy, catheters for chemotherapy infusion, etc). New methods of radiotherapy (neutrons, pi mesons, interstitial use of isotopes, etc), new machinery for better delivery of radiotherapy, and drugs which can protect or enhance radiation effects have been developed. Still, these two modalities can be useful mainly in the local and regional treatment of cancer.

Chemotherapy continues to develop, with new drugs and new combinations of drugs being discovered which are improving cancer treatment. While there has been continued slow progress over the last 5 years, the rapid progress during the previous 10–15 years in the treatment of highly replicative and sensitive malignancies is not continuing. It is now apparent that further progress with chemotherapy will probably depend on a greater understanding of the metabolic processes of cancer cells and the differences between these and normal cells. In addition, there are the problems of selectivity and drug delivery. Clearly, cancer cells are more like than unlike normal cells with respect to sensitivity to current chemotherapeutic agents. There is also little evidence of selectivity of effect or in the delivery of drugs to neoplastic tissue. Given the systemic administration of chemotherapeutic agents, one is also left with the systemic exposure of normal cells to these drugs. It is well-known that we

---

[1] Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, Frederick, MD.

[*] *Reprint requests to:* Robert K. Oldham, MD, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, PO Box B, Frederick, MD 21701.

have discovered many chemotherapeutic agents which are highly cytolytic, but the problems of normal tissue toxicity, drug delivery, and tumor cell resistance remain with us (2,3). Thus, cancer remains a systemic problem which requires further systemic approaches for more effective treatment.

The scientific base is now firm for the establishment of the fourth modality, the use of biologicals and biological response modifiers in the treatment of cancer. Historically, there was an attempt to establish immunotherapy as the fourth modality based on a considerable body of experimental evidence that alteration of the immune system could be an effective cancer treatment modality in animal models. These data seemed to indicate that both specific stimulation of the immune response with antigen-bearing tumor cells and nonspecific immunological stimulation with bacteria, viruses, and other adjuvant-type compounds could enhance the existing immune response in animals and prevent recurrence or delay growth of experimentally transplanted tumors. While the effects were reproducible under specific circumstances, immunotherapy was not strikingly effective in animals bearing palpable tumors. Given the observation that immunotherapy was more effective with smaller tumor burdens, investigators began to study both specific and nonspecific immunotherapy as treatment for minimal residual disease. Although the concept was established that the treatment of animals with minimal residual disease could be analogous to the postsurgical treatment of cancer in humans, this analogy was often stretched to the limit, in that immunotherapy in animals was often begun on the day of (or within a day or 2 of) the tumor transplant. Often the transplant consisted of a very small number of tumor cells (one to 1000). In many of these studies and in studies where the tumor was surgically resected and no evident disease remained, the effects of immunotherapy were reasonably reproducible and were most beneficial when the tumor mass was $\leqslant 10^6$ cells.

These experimental results developed into the dogma that immunological manipulation or immunotherapy could work *only* when the tumor cell mass was imperceptible (4). This raised definite difficulties for the immunotherapy of human malignancies because the tumor cell mass is generally at least two orders of magnitude greater than 10^6 cells at clinical diagnosis. Even in the postsurgical adjuvant setting, the timing of recurrence would seem to indicate that larger numbers of cells were probably present, although clinically imperceptible, than immunotherapy would likely be able to cure.

Despite the difficulties obvious in the experimental results with animal tumor models, clinicians began to intensively investigate immunotherapy in the 1970s. As the results of these studies were reported a general trend developed. While a small, initial nonrandomized trial would often be reported as being positive in a preliminary report, follow-up of those patients would often reveal that the study was, indeed, negative. Often the preliminary results of the smaller studies prompted the initiation of larger, randomized, controlled studies to confirm or deny the efficacy of a particular immunotherapeutic regimen in a particular cancer. While some of the controlled studies were positive, most yielded marginal or negative results which contributed to the negative attitude of most clinicians toward immunotherapy by the end of the 1970s. Many of these studies are summarized in a recent book on the immunotherapy of cancer (5). However, all immunotherapy studies were not negative. There have been several randomized, controlled studies in which positive results have been seen and these studies raise questions about the adequacy of design in other clinical immunotherapy studies (3,4).

Why did immunotherapy fail to establish itself as the fourth modality? A major factor was the lack of purity of the reagents for immunotherapy. Many of the nonspecific approaches involved the use of complex chemicals, bacteria, viruses, and poorly defined extracts in an attempt to "stimulate" the immune response. Even for specific immunotherapy, where the rationale was somewhat stronger, most investigators used cells or cell extracts to try to stimulate the specific immune response without any molecular definition as to the actual stimulating entities (such as tumor-associated antigens) which might have been involved in the treatment. With these poorly characterized reagents, the problems of variability of experimental procedures and the lack of efficacy were hardly surprising.

Biological control mechanisms should be envisioned on a much broader basis than the immune system. While immunotherapy remains a subcategory of biological therapy, there are numerous additional possibilities for the control of neoplastic growth and metastasis through the use of growth and differentiation factors, through the use of synthetically derived molecular analogs, and through the pharmacologic exploitation of biological molecules, which involve a much broader range of approaches than those previously considered in immunotherapy.

Why is it now possible to define a fourth modality of cancer treatment? Major technological advances have been made in the last 5-10 years, which give a strong basis for this modality. First, advances in molecular biology have given scientists the capability to clone individual genes and thereby produce huge quantities of highly purified products of the mammalian genome for analysis. These protein products of cloned genes can be made as essentially pure materials and the biological effects of these molecules can be investigated rather precisely. Unlike the extracted and purified biological molecules, which were always available in small quantities as semipurified mixtures after rather laborious attempts at purification, the products of cloned genes have a level of purity on a par with drugs and can be analyzed alone or in combination as to their effects in cancer biology. In addition, our recent progress in nucleic acid sequencing and translation, protein sequencing and synthesis, isolation and purification of biological products, mass cell culture, and the "genetic engineering" of these genes has given us the opportunity to manufacture and test these highly purified biologicals and to alter them at the nucleotide or amino acid level to manipulate and optimize their biological activity.

A second major advance was the discovery of hybridomas. A major limitation for the use of antibodies was the inability to make high-titer specific antisera and to define these preparations on a molecular basis. With the hybridoma-monoclonal antibody technology, one can now make these immunoglobulin reagents with the same level of molecular purity as that for cloned gene products and for drugs. These monoclonal antibodies are powerful tools in the isolation and purification of tumor-associated antigens, lymphokines/cytokines, and other biological molecules which can be used in biological therapy. The advances in molecular biology and hybridoma technology have made the previous techniques for the discovery and purification of biological molecules virtually extinct. These new techniques make possible the discovery and testing of biological molecules within months that previously might have taken years to accomplish even if it were possible.

Finally, technological advances in equipment and computers have been basic both to our technology for isolating and purifying biological molecules and to the speed with which this can be done. We now have the capability to construct nucleotide or amino acid sequences to fit any message that we are able to decipher from a biological molecule. While this synthetic capability is currently limited to smaller genes and gene products, the techniques are rapidly becoming available where analysis and construction of nucleotide sequences will occur in an automated way, making enormously complex molecules possible to manufacture and investigate.

In response to the recent advances, it was recognized that a focused approach by the National Cancer Institute (NCI) in this area might improve the transfer of technology from the laboratory to the clinic. The Biological Response Modifiers Program (BRMP) was established in 1980 to provide support in this area. The development of this program has been recently reviewed (6,7).

Biologicals and Biological Response Modifiers

Biologicals are products of the mammalian genome. Biological response modifiers are agents and approaches whose mechanisms of action involve the individual's own biological responses. Biologicals and biological response modifiers can act in the following ways:

(*a*) increase the host's antitumor responses through augmentation and/or restoration of effector mechanisms or mediators of the host's defense and/or decrease the component of the host's reaction that may be deleterious;

(*b*) increase the host's defenses (natural biological or the synthetic derivatives thereof) as effectors or mediators of an antitumor response;

(*c*) augment the host's responses to modified tumor cells or vaccines, which might stimulate a greater response by the host or increase tumor cell sensitivity to an existing response;

(*d*) decrease the transformation and/or increase differentiation (maturation) of tumor cells; and

(*e*) increase the ability of the host to tolerate damage by cytotoxic modalities of cancer treatment.

While many of the approaches described above involve the augmentation of host responses, an understanding of the biological properties of the molecules of the immune response and of the host responses related to growth and maturation will assist in the development of specific molecular entities which have direct actions on both host responses and tumor cells. Thus, one can visualize the development of biological agents with host-modifying and direct cytostatic, cytolytic, or maturational effects on tumor cells.

Now that it is clear that the mechanisms are available for the discovery and development of biological molecules and response modifiers for cancer treatment, how may these best be put into clinical practice? Clearly, it is important to dispel the historical dogma of immunotherapy. These molecules can have activity on clinically apparent disease, and their testing is not restricted to situations where the tumor cell mass is imperceptible (4). Thus, the clinical trial designs necessary to test new biologicals and new biological response modifiers can be similar to those used previously for other modalities of cancer treatment, as long as one is sensitive to the need to measure both the pharmacokinetics of the biological or biological response modifier and the biological responses affected by these approaches. Thus, testing has been initiated and is continuing for the interferons (alpha, beta, and gamma), lymphokines/cytokines, growth and maturation factors, monoclonal antibodies and immunoconjugates thereof, and adoptive cellular therapy. Since the latter category is being reviewed elsewhere in this volume, this paper will concentrate on the molecular substances which may have therapeutic activity.

Clinical Approaches

Given the variability of cancer's clinical presentation, it is not surprising that randomized trials of nonspecific and specific immunotherapy, as translated from animal models, have not uniformly reduced the incidence and growth of recurring cancer. The recent scholarly debate between Hewitt (8) and Herberman (9) has illuminated many of the problems and prospects in the area of tumor immunobiology. Naturally occurring cancer arises from one cell or a few cells under some carcinogenic stimulus in a particular organ. In humans, these initial foci of cancer cells may grow over very long periods of time (from 1% to 10% of the human lifespan) before there is any clinical evidence of the disease. Dissemination of cells from the initial foci may occur at any time during the development of the primary tumor. Thus, metastasis can occur over a long growth period, allowing complex biological interactions to occur during the process.

By contrast, experimentally induced cancer is an artificial situation in many respects. Not only are tumor cells injected into normal animals, thereby circumventing the influence of environmental or genetic factors which may be operative in the natural host during tumor development, but the injection also represents a single instantaneous point source for a defined tumor load. Regardless of whether that tumor load is 10^3 or 10^8 cells, it is being placed artificially into a single site and allowed to grow and metastasize from that artificially selected single site. Thus, these transplantable cancers are not analogous to clinical cancer.

Clinical trials with biological response modifiers require a different approach from that traditionally used to test new drugs. Whereas drugs are initially tested for toxicity (phase I) and then for antitumor activity (phase II), biological response modifiers must also be tested for their ability to modify the appropriate biological response. The maximum tolerated dose and the maximum biological response-modifying dose (phase I) should be established. Both doses may need to be tested in phase II efficacy trials because more of a particular biological response modifier may not necessarily be better therapeutically (10,11).

Interferons

Interferons are small, biologically active proteins currently classified into three types: alpha, beta, or gamma. The alpha interferons consist of a family of interferon molecules that represent the products of several genes. The number of beta and gamma interferon molecules produced naturally by human cells is not known with certainty, but there appear to be fewer molecular types of these interferons than of alpha interferon.

Each interferon type has distinctive capabilities in altering a variety of immunological and other biological responses. As a class, the interferons appear to have some growth-regulating capacity in that antiproliferative effects are measurable with in vitro assays and in animal model systems. The relative efficacy of the mixtures of natural interferons that occur after virus stimulation as compared to the cloned interferons remains to be precisely determined. Because there may be > 20 interferon molecules (and hundreds of recombinant hybrids thereof) and because attempts have already begun at recombining the different molecules into mixtures of interferon as well as at altering individual interferon molecules in specific ways, the range of biological activities of the interferons as antiviral agents, as immunomodulating agents, and as antiproliferative agents may be very broad.

In addition to high antiviral activity, the interferons have profound effects on the immune system. Relatively low doses will enhance antibody formation and lymphocyte blastogenesis, while higher doses will inhibit both of these functions. Low to moderate doses may inhibit delayed hypersensitivity while enhancing macrophage phagocytosis and cytotoxicity, natural killer (NK) activity, and surface antigen expression. Interferons also prolong and inhibit cell division, having this effect on almost every cell system studied, whether transformed or normal. In addition, interferons stimulate the induction of several intracellular enzyme systems with resultant profound effect on macromolecular activities and protein synthesis. All of these functions have been documented in murine systems, but complete dose-response effects for all types of interferons in these cellular activities have not been thoroughly investigated in either mouse or man. Most of the work to date has been done with alpha interferons. It would appear that beta and gamma may have similar effects on many systems, but relative potency may vary.

With respect to cancer therapeutics, it is still unclear whether the interferons work primarily by their antiproliferative activity or through alterations of immune responses. What is clear from the current preclinical and clinical studies is that the interferons have *antitumor* activity (10,11). Clinical activity has been seen most reproducibly with a variety of lymphomas, but responses in many other tumor types have been seen (4,11). The best dose, schedule, route of administration, and type of interferon need to be determined by further efficacy studies, and the use of interferon in combination with other anticancer agents is just beginning.

Thymic Factors

It has been known for years that thymic extracts have biological activity on cells in the immune system (12). Although a variety of thymic extracts have been investigated, two preparations have received the most attention in the laboratory and the clinic: thymosin fraction 5 and thymosin α-1. Thymosin fraction 5 is an extract containing a variety of thymic polypeptides, and thymosin α-1 is a synthetic polypeptide component which is also present in many thymic extracts. Because thymosin α-1 can be produced synthetically, it is the most reproducible thymosin preparation available for clinical studies. Many of these thymic preparations have been shown to enhance, and some have been shown to suppress, immune responses in both intact and thymectomized animals. Many investigators have reported that the thymosins can correct immunodeficiency states, both natural and laboratory-induced. There have also been reports that thymic factors can augment suppressed or depressed T-cell responses in patients with cancer. Similar to interferons, thymic extracts represent a family of compounds. Studies are in progress with thymosin fraction 5 and with thymosin α-1 to determine their efficacies in preclinical models and in patients with cancer (13,14).

Lymphokines and Cytokines

Many of the biologicals which will be tested as response modifiers are cell products (cytokines) of lymphocytes (lymphokines) or direct cytotoxic factors of activated lymphocytes (lymphotoxins) or macrophages (cytotoxins). The lymphokines have a specific ability to regulate certain components of the immune response, which may be useful in altering the growth and metastasis of cancer in man. There are early indications of the specific ability of lymphokines to alter immune response in ways that may be beneficial in the host-tumor interaction. For example, it is possible that certain lymphokines may augment the ability of T cells to respond to tumor-associated antigens, and others may induce higher responsiveness with respect to B-cell activity in cancer patients. Additionally, lymphokines which decrease suppressor functions of the immune system may be useful in enhancing immune responses through a lessening of suppressive effects. Another specific use of lymphokines may be in the pharmacologic regulation of tumors of the lymphoid system. While many of these tumors are considered to be generally unresponsive to normal growth-controlling mechanisms mediated by lymphokines, it is possible that large quantities of pure lymphokines administered pharmacologically or the use of certain molecular analogs of these naturally occurring lymphokines may be useful in the treatment of lymphoid malignancies. A further use of lymphokines may be to manipulate the immune response in vitro to produce products or cells which may subsequently be used therapeutically in vivo.

Interleukin 1 (IL-1), originally known as lymphocyte-activating factor, is a macrophage-derived cytokine that was identified originally as a result of its nonspecific enhancing effect on murine thymocyte proliferation. Both IL-1 and viable macrophages are necessary for the initial step in activation of specific T cells and the process of in vivo production of effector T cells.

IL-1, in its purest form, stimulates the production of Interleukin 2 (IL-2), also known as T-cell growth factor. The assistance of macrophages is also required in this step. Cloning of IL-1 and IL-2 will soon make large quantities of highly purified materials available for further studies.

A lymphotoxic product of antigen- or mitogen-stimulated leukocytes was the first lymphotoxin described and is the most investigated. Lymphotoxin may be the principal effector of delayed hypersensitivity and, although conflicting data have been reported, may also be involved in the cytotoxic reactions of T-cell-mediated lysis and K-cell lysis. Depending upon the type of tumor cell involved, the in vitro effect of lymphotoxin may be either cytolytic or cytostatic. Mouse tumor cells are frequently killed by homologous and heterologous lymphotoxins, whereas in other species, reversible inhibition of tumor cell proliferation is more common. Presumably, if lymphotoxin is involved in the lytic activation of T cells, it is either one component of a multicomponent attack or is presented by the effector cell in a form likely to cause target cell destruction.

Human lymphotoxin has been produced for peripheral blood lymphocytes or tonsillar lymphocytes by stimulation with phytohemagglutinin or concanavalin A, and it has been harvested from supernatants of lymphoblastoid cell lines which constitutively produce small amounts of lymphotoxin (15). Isolation of human lymphotoxin for in vivo studies is complicated by the occurrence of at least five major species and several subspecies which result from the association with other components and subsequent spontaneous degradation to lower molecular weight forms.

In vivo studies with human tumor cells have used intralesional injection of crude extracts containing lymphotoxin activity to directly observe the effects on the tumor cells. With accessible tumors such as melanomas and prostatic and bladder carcinomas, local injection may be efficacious. For other types, however, systemic treatment must be considered.

Combined treatments of lymphotoxin, local or systemically administered, with other antitumor agents may be more valuable than lymphotoxin alone. Since lymphotoxin alone exhibits little toxicity, lymphotoxin may be used to increase the effects of chemotherapeutic agents without added toxicity. What may be equally important in this regard is the ability of lymphotoxin to inhibit chemical- and radiation-induced neoplastic transformation. In these studies using a hamster transformation model, lymphotoxin was found to be >20 times more effective as an anticarcinogenic agent than as an antitumor agent (16). Lymphotoxin used as an adjunct to chemotherapy may permit higher levels of effective but potentially carcinogenic agents to be used with less risk of producing a second malignancy.

Lymphotoxin antitumor activity may be potentiated when it is given with other lymphokines such as macrophage-activating factor, interferon, and IL-2. Lymphotoxin directly inhibits the growth of some tumor cells and also renders these cells more susceptible to NK-mediated lysis. Since interferon enhances the activity of NK cells, lymphotoxin and interferon given together or in sequence may result in more NK-mediated killing than that obtainable with either agent alone.

It has been estimated that >100 biological molecules have already been described as lymphokines (17). Clearly, several lymphokines such as lymphotoxins, macrophage-activating factor, IL-2, and possibly tumor necrosis factor merit evaluation as antitumor agents since each, through its own distinct mechanisms, may contribute to tumor control. Such studies require quantities of material sufficiently pure to exclude contributions by other factors to permit definitive evaluation of each lymphokine/cytokine. Larger-scale studies will require standardized preparations in quantities best obtained through genetic engineering with the use of sensitive and rapid assay procedures suitable to monitor production, purification, and bioavailability.

Monoclonal Antibodies

Antibody therapy for cancer in animal tumor models and in man has recently been reviewed (18,19). There have been preliminary results reporting the use of monoclonal antibody in a few solid tumors such as melanoma and gastrointestinal cancer, but the larger number of patients treated thus far have had leukemia or lymphoma. Antibody trials in human T-cell leukemia and lymphoma have been attempted using monoclonal antibodies with T-cell specificity. There are now a large number of antibodies (eg, T101, OKT-1, and anti-Leu-1) that react with most circulating T cells. While such antibodies have been effective in causing sharp drops in the circulating wbc counts, the therapeutic effects were brief, lasting sometimes only hours before the leukemic cell count increased to previous levels. However, occasional patients with cutaneous T-cell lymphoma (CTCL) have had a partial response in the skin lesions and lymph nodes, accompanied by a sustained reduction in circulating normal and malignant T cells, with repeated intermittent monoclonal antibody therapy (20). Some investigators believe that the reticuloendothelial system plays a major role in these responses where antibody coating of circulating cells may be important in removing circulating leukemia cells. Of interest in this regard has been the lack of effect on bone marrow leukemic cells during antibody therapy, even with an effective coating of the tumor cells by antibody (19).

Further studies are underway with the use of anti-T-cell reagents in T-cell leukemias, lymphomas, and T-antigen-bearing chronic lymphocytic leukemia (CLL). The BRMP is currently supporting four clinical trials with monoclonal antibodies in these disorders. These trials should be of value in defining the role of anti-T-cell monoclonal antibody alone in the treatment of these leukemias and lymphomas. Preliminary results using T101 in ten patients with CLL and in seven patients with CTCL have been reported (20). These studies have confirmed the binding of T101 antibody to circulating and bone marrow cells. No in vivo binding was seen in malignant lymph nodes for patients treated with 10–50 mg of antibody iv, but this is being studied at higher doses. However, patients with skin infiltration have demonstrated in vivo binding of the antibody to neoplastic cells infiltrating the skin. Antigenic modulation, where the antigen disappears from the cell surface by shedding or internalization, was noted for cells in the blood, bone marrow, and skin. For this particular antigen, modulation occurred within 2 hours after the administration of antibody and antigen and usually reappeared within 24 hours. These studies indicate that anti-T-cell monoclonal antibody can be safely administered, will bind to circulating cells bearing this antigen with some brief drop in the cell count, will bind to leukemia cells in the marrow and skin as demonstrated by immunoperoxidase or flow cytometry, and will rapidly induce antigenic modulation. In addition, there has been some clinical toxicity with the infusion of T101. Pulmonary toxicity was observed with infusion rates > 5 mg/hour and most clearly when they were > 10 mg/hour. This was manifest clinically by acute dyspnea and mild wheezing. Clinical and laboratory monitoring seemed to indicate evidence of lymphocyte agglutination or trapping in the lungs after antibody treatment (20).

A more specific approach in the use of monoclonal antibody therapy has been described by Miller et al (21). One of their patients with a poorly differentiated B-cell lymphoma became resistant to cytotoxic drugs and to interferon. An anti-idiotype monoclonal antibody was developed which was specific for the idiotype on the surface membrane of this patient's lymphoma cells. Circulating (IgM) idiotype was detected prior to therapy, but with continued monoclonal antibody therapy the idiotype level fell and then disappeared from the circulation. Subsequent to the appearance of circulating anti-idiotypic antibody, a clinical response began which became complete during the course of continued intermittent anti-idiotype therapy. This complete response is stable at 2 years and subsequent patients are being similarly treated with encouraging results (R. Levy and R. Miller, personal communication). Miller and Levy have postulated that the normal T cells present in the neoplastic lesions might induce the destruction of antibody-coated tumor cells in this clinical response. A direct antiproliferative or growth regulatory ("switch off") effect could also be postulated.

Although large numbers of monoclonal antibody trials are being initiated, only a few trials have been underway for a sufficient period of time to have reported any results. Some of the trials where results have been reported and some where patients are still accruing are listed in table 1. Based on these preliminary trials using mouse-derived monoclonal antibodies, it is clear that these mouse immunoglobulin reagents can be given to man without severe acute toxicity. Clinical trials investigating different doses and schedules of administration are underway to determine the optimal way to give iv antibody alone in

TABLE 1.—Monoclonal antibody serotherapy trials*

Institution	Disease	Monoclonal antibody
Dana-Farber Cancer Center	Lymphoma	Ab89
Dana-Farber Cancer Center	Acute lymphocytic leukemia	J-5
Stanford University	T-Leukemia	Anti-Leu-1
Stanford University	CTCL	Anti-Leu-1
Stanford University	Lymphoma	Anti-idiotype
University of Pennsylvania	Gastrointestinal tumor	17-1A
University of California, San Diego	CLL, CTCL	T101
NCI-BRMP	CLL, CTCL	T101
University of California, San Diego	Melanoma	Anti-p97, anti-p240
Fred Hutchinson Cancer Center	Melanoma	Anti-p97
NCI-BRMP	Melanoma	9.2.27
University of California, San Diego	Colon	065

* References for these studies are available in ref 19.

these phase I trials. Certain conclusions can be drawn based on these preliminary studies (table 2). A major difficulty with antibody alone appears to be that of antigenic modulation. Thus far, antigenic modulation has been observed more with the antigens on hematopoietic cells than with those on solid tumors. While modulation may be a limiting problem for antibody alone, it may be a positive factor for immunoconjugate therapy, where the incorporation and toxicity of immunoconjugates into the tumor cell may occur prior to or during modulation. In fact, it has been postulated that modulation may be a mechanism by which an immunoconjugated molecule could gain entrance into the cell. In addition to our studies with T101, we have initiated a trial with the 9.2.27 monoclonal antibody reacting with a 250-kd antigen on the melanoma cell surface. This antigen is not shed in significant quantities into the supernatant of cultured melanoma cell lines and is not detectable in the serum of patients with melanoma. In addition, no evidence of modulation in the presence of antibody has been seen. Approximately ten patients have been studied and doses up to 500 mg of this murine-derived monoclonal antibody have been given iv. Because this antigen is not found on any circulating cells, no evidence of pulmonary toxicity has been seen. With doses ≥ 10 mg, excellent evidence of tumor cell labeling with iv administered antibody has been seen. Cutaneous nodules show excellent staining of the melanoma cells when removed 24 hours to 4 days after the infusion of 9.2.27 at doses ≥ 10 mg. A clear dose response (concentration × time) relationship has been seen. Smaller doses of antibody show staining primarily of cells near the vasculature in the melanoma nodule while larger doses show greater diffusion of antibody out into the interstices of the melanoma nodule. Using an immunoperoxidase technique, excellent specificity has been seen in the staining of these nodules by circulating antibody. Total saturation has not been achieved at these doses since added antibody in vitro has always demonstrated additional staining of the melanoma cells. Thus far, this antigen has been demonstrated in $> 90\%$ of the melanoma biopsies and no evidence of heterogeneity within individual nodules or among different nodules from the same patient has been seen. Trials with labeled antibody are underway which should clarify the in vivo distribution of this antibody in patients with melanoma. While therapeutic effects have not been dramatic, the evidence for specific trafficking of the antibody to the melanoma nodules is very encouraging and lends great strength to the concept of using monoclonal antibodies as targeting agents for immunoconjugates carrying cytotoxic drugs, toxins, or radioisotopes to the cancer cell.

The use of monoclonal antibody and its conjugates in the treatment and radioimaging of cancer is in a very early stage. While it is clear that much needs to be done to clarify many of the issues surrounding the use of antibody alone or conjugates of the antibody with other toxic substances, it has already been demonstrated in both animal tumor models and in man that antibody alone and antibody conjugated with drugs, toxins, and radioisotopes can have therapeutic effects. The potential for monoclonal antibodies in cancer therapeutics is enormous given the specificity that is inherent to the antibody-antigen reaction. Xenogeneic monoclonals have the advantage of recognizing tumor cells which may be relatively

TABLE 2.—Monoclonal antibody (MoAb) serotherapy trials: conclusions

1. Iv murine-derived MoAb can be given safely by prolonged infusion (> 1 hr) without immediate serious side effects.
2. Pulmonary toxicity (dyspnea, bronchospasm, and chest pain) and hypotension have followed rapid infusion (> 5 mg/hr) of anti-T MoAb in the presence of circulating cells bearing the antigen.
3. Dosages from 1 to 1500 mg have been given safely with careful monitoring.
4. Transient reductions in circulating T cells are usually seen with anti-T MoAb but a sustained decline in leukemic cells requires multiple MoAb treatments over several weeks.
5. CTCL skin lesions and lymph nodes can regress following anti-T MoAb treatment.
6. There has been no reduction in the bone marrow leukemia cells.
7. Antigenic modulation sometimes occurs following MoAb treatment and has been very common in CLL and T-cell malignancies with anti-T MoAb.
8. Free antigen may be detected in the serum following MoAb treatment.
9. Antimouse antibodies may develop following MoAb therapy.
10. Serum sickness has been documented as an infrequent complication of MoAb therapy.
11. There is considerable variation with respect to toxicity, bioavailability, and activity related to immunoglobulin class, antigen, and distribution of tumor.

nonimmunogenic in man but which carry antigenic structures which are immunogenic in the xenogeneic species. Extensive phase I trials are needed to document the biologic and the toxic effects of antibody alone before antibody conjugate studies are done in man. These studies should investigate a wide dose range of antibody administered by different schedules utilizing reagents from different xenogeneic sources and of different antibody classes. These antibody-containing reagents should be prepared in a highly purified state, and trials using antibody fragments should also be initiated to improve our understanding of the role of antibody alone in man. Subsequent studies may utilize monoclonal antibody from human sources when these are available.

Extensive animal model studies with immunoconjugates should be done before in vivo trials with these reagents are initiated in man (18,22,23). The use of isotope-labeled antibody has enormous potential for the detection and treatment of cancer (24). There are still significant problems with these conjugates in terms of potential toxicity for the organs nonspecifically accumulating radioactivity from the conjugate or its products, and any nonspecific binding of isotope-labeled conjugates will represent a significant clinical problem for the use of these conjugates in therapy. By contrast, toxins and especially drugs may be more approachable with regard to early clinical trials. There is considerable experience with drugs and their clinical toxicity. The hypothesis that lower levels of drug toxicity and greater antitumor activity may be seen by virtue of increased specificity of the drug-antibody conjugate remains to be demonstrated in man. With toxins, the potential for using a virtually nontoxic A chain in an immunoconjugate to carry this toxin to its site of action inside the cell is particularly appealing. An interesting model in man for the *early* use of drug and toxin immunoconjugates is the bone marrow transplantation situation. Here, one can treat the bone marrow for remaining tumor cells with an immunoconjugate and reinfuse the bone marrow after appropriate cleansing with very little risk of infusing significant quantities of the antibody conjugate in vivo. Antibody plus complement has already been reported to be efficacious in this clinical setting (25). Antibody or immunoconjugates to specific normal T cells may be useful in eliminating graft versus host reactions after allogeneic bone marrow transplantation. This may allow for wider application of transplantation requiring less precise matching of donor and recipient. In this way, one may be able to assess, in a preliminary way, the value of immunoconjugates in man even before the therapeutic/toxic ratio of these reagents has been extensively investigated in phase I toxicity trials with infused antibody. While much work needs to be done to define the role of monoclonal antibody in the treatment of cancer, the future seems very bright, and this is the area of cancer biology that deserves the greatest emphasis for the development of new therapeutic reagents in the coming years.

Tumor Antigens and Active Specific Immunotherapy

There has been a substantial effort to actively immunize autochthonous or syngeneic hosts with irradiated or chemically modified tumor cells in an attempt to use active specific immunotherapy. Inherent in this approach is the assumption that tumor cells express immunogenic tumor antigens. Treatment of tumor cells with a variety of unrelated agents such as irradiation, mitomycin, lipophilic agents, neuraminidase, viruses, or admixtures of cells with bacterial adjuvants produced nontumorigenic tumor cell preparations that are immunogenic upon injection into syngeneic hosts.

Recently, a re-evaluation of the procedures of active specific immunotherapy using BCG-tumor cell (antigens) vaccines has been undertaken in an animal model using a syngeneic hepatocarcinoma in an inbred strain of guinea pigs. Investigations of several variables of vaccine preparation, such as ratio of BCG organisms to viable, metabolically active tumor cells, the procedures of cryobiologic preservation, and the irradiation attenuation of cells, have resulted in the development of an optimal nontumorigenic BCG plus tumor cell vaccine, as well as a most effective regimen for the treatment of both micrometastatic and limited macrometastatic disease (26–28).

Insight into the mechanism of active specific immunotherapy was gained from morphologic studies in the guinea pig model which revealed that host cell-mediated hypersensitivity reactions occurred at the sites of pulmonary metastases in the immunized guinea pigs. These nodules were infiltrated by a predominantly mononuclear cell population made up of lymphocytes and cells of the macrophage-histiocyte series. This infiltration disrupted the typical compact architecture of tumor foci. Presumably, some of the infiltrating lymphocytes-monocytes were sensitized to tumor-associated antigens. These effector cells, while not only being directly tumoricidal, also release lymphokines/cytokines in the presence of specific antigens. These factors are chemotactic for mononuclear cells and initiate inflammatory tissue reactions which may contribute to the disruption of the nodules (28).

The nature of this anatomic alteration in metastatic nodules that accompanies active specific immunotherapy was explored further by use of a specific monoclonal antibody as a probe to assess vascular permeability within these tumor models (29,30). Immunohistologic analysis of antibody distribution showed that significantly more antibody accumulated in tumors from chemotherapy-treated and/or vaccinated guinea pigs than in comparable tumors from untreated guinea pigs. This is a key finding in that research into mechanisms by which tumors evade the immune system have focused primarily on the poor immunogenicity of spontaneous tumors or the immune competence of the host. Less attention has been given to the possibility that the anatomic characteristics of tumor foci restrict drug or host interactions, thus protecting tumors not only from immunotherapy but from other forms of treatment as well (3).

Several reports suggest that the regulation of the blood supply to neoplastic tissues may be different from that of the host tissues invaded by the tumor. This biologic state of the tumor metastatic nodule contributes to the fact that blood-borne substances, such as chemotherapeutic agents, monoclonal antibodies, and immune effector cells, would encounter this vascular barrier, thus limiting their access to all portions of the tumors. Such vascular barriers may provide an environment in which some tumor cells survive blood-borne chemotherapeutic and biologic agents. In this respect, solid tumor nodules may serve as "pharmacologic sanctuaries," allowing even drug-sensitive tumor cells to continue to grow (3). It is reasonable to postulate that successful therapy must include a process that both overcomes these anatomic barriers and delivers cytotoxic agents to the tumor(s). One host response that could potentially achieve this goal, as demonstrated in this guinea pig model, is an induced cell-mediated immune reactivity resulting from active specific immunotherapy. Another response might involve lymphokines/cytokines (such as tumor necrosis factor), macrophage cytotoxic factors, and activated complexes (such as those generated by plasma perfusion over protein A columns). The delivery of these substances to tumor nodules might increase the vascular permeability and increase the access of antibody, immunoconjugates, drugs, and activated cells to the cancer cells.

To test this hypothesis, guinea pigs with disseminated cancer were treated with active specific immunotherapy alone, chemotherapy alone, or a combination of the two (30). These studies demonstrated that strategically timed chemotherapy *subsequent* to immunotherapy can effectively double the number of survivors attainable with immunotherapy alone. Furthermore, it has been shown that the synergistic effects obtained by combining immunotherapy with chemotherapy are not drug-specific. These results suggest a new basis for active specific immunotherapy in the treatment of solid tumors. Inflammatory disruption of anatomic barriers of metastatic nodules combined with strategically administered chemotherapy or biological therapy may prove to be useful in the design of future clinical trials in man.

A major limitation of active-specific immunotherapy is the source and availability of the tumor antigens. As presently established, the necessity of adhering to a strict protocol for whole-cell vaccine preparations (which may differ among different tumors) constitutes a major limitation of this procedure for the clinic. While the present vaccine preparations must contain viable, nontumorigenic cells prepared from individual tumors, it is possible that in the future monoclonal antibody-defined purified tumor antigens would be available for large-scale immunizations. With these, a procedure can be visualized which would include antigen purification and characterization followed by genetic engineering of the antigen for vaccine production. All of these technologies are at hand and specific tumor antigen preparations will soon be available for clinical evaluation.

Immunomodulating Agents

Since the early 1900s, immunotherapy with bacterial or viral products has been utilized with the hope of stimulating the host's immune response (4). Many of the stimulants are nonspecific in the sense that they may generally activate the immune response, rather than activating only the specific component directed toward the tumor. These agents have been useful as adjuvants and as nonspecific stimulants in animal tumor models. However, human trials have been disappointing, and it is currently believed that a major difficulty has been our lack of understanding of tumor-associated antigens and tumor cell heterogeneity with respect to the attempted stimulation of the immune response in humans. Clearly, in the animal tumor models, specific requirements for immune stimulation are much better defined. The translation of that immune stimulation to humans has been very difficult, given the lack of information on the level of immunity and other factors in human cancer. It may be possible that purified components of bacterial cell walls, fungi, parasites, viruses, or specific chemicals (table 3) will lead to the development of more effective adjuvants or stimulants of the immune response for use in association with the tumor-associated antigens in active specific immunotherapy.

Perspectives

What should we expect from this fourth modality of treatment in this decade? At the outset, it is clear that we now have much more powerful tools for improving cancer therapy in the future. The biological approaches described in this review represent not only approaches to therapy but also powerful tools for the dissection of the major problems in cancer biology. We now have the techniques to decipher the major problems in cancer biology down to the genetic level. The development of these techniques, along with the recognition that biologicals can provide, for the first time, specificity in cancer treatment, supports the belief that new and highly effective approaches are likely to develop through this fourth modality. As with the other three modalities, biological therapy should not stand alone. It provides an additional technique which may work most effectively in combination with surgery or radiotherapy to decrease the local and regional tumor or with chemotherapy to reduce the systemic tumor burden. It may work very effectively with radiotherapy in the sense of using biological reagents to target radioisotopes specifically to the tumor site or with traditional chemotherapy with respect to enhancing the specificity of drug delivery.

Recent observations on drug resistance have focused on mechanisms at the cellular or clonal level. Multidrug resistance with a genetic linkage is being postulated as a major reason for resistance to traditional chemotherapy. However, most patients with cancer demonstrate resistance (insufficient clinical tumor response) to the first chemotherapeutic agent or agents they receive, a finding which does not fit well with the concept of *drug-induced* resistance. Certainly, cancers may be derived from primary clones that were intrinsically drug-resistant, or cells may arise as resistant clones by spontaneous mutation. However, another mechanism for drug resistance may relate to drug delivery. In human tumors, the initial resistance seen may relate more to the inability of the drug to reach all of the tumor cells in adequate concentrations with an adequate exposure time rather than the tumor cells being intrinsically drug-resistant (3).

Most human cancers that are susceptible to chemotherapy are highly replicative, very metabolically active tumors (ie, poorly differentiated lymphomas, testicular, and small cell lung cancers). Many of these tumors have high cell viability and may secrete various substances into the tumor milieu. These conditions may favor the diffusion of chemotherapeutic agents into the interstitial space around the tumor cells and provide high concentrations of the drug to the metabolically active tumor cells. By contrast, slowly growing tumors with much central necrosis may represent "pharmacological sanctuaries," in terms of drug delivery (3). Slowly growing tumors (eg, lung, breast, colon, and prostate cancers) may be resistant because of lack of delivery of the chemotherapeutic agent and lack of prolonged exposure at adequate concentrations rather than being a problem of intrinsic drug resistance. Studies using dissociated tumor cells tested in vitro for drug resistance or studies using drug-specific antibody which can be localized by immunoperoxidase

TABLE 3.—Biologicals and biological response modifiers

Immunomodulator and/or Immunostimulating Agents	
Alkyl lysophospholipids (ALP)	Lentinan
Azimexon	Levan
BCG	Muramyldipeptide (MDP)
Bestatin	Malic anhydride-divinyl ether (MVE-2)
Brucella abortus	Mixed bacterial vaccines
Corynebacterium parvum	N-137
Cimetidine	*Nocardia rubra* cell wall skeleton (CWS)
Sodium diethyldithiocarbamate (DTC)	Picibanil (OK 432)
Endotoxin	Prostaglandin inhibitors (aspirin, indomethacin)
Glucan	Thiobendazole
"Immune" RNAs	Tilorones
Therafectin	Tuftsin
Krestin	
Interferons and Interferon Inducers	
Interferons (α, β and γ)	*Brucella abortus*
Poly IC-LC	Viruses
Tilorones	
Thymosins	
Thymosin α-1	Other thymic factors
Thymosin fraction 5	
Lymphokines and Cytokines	
Antigrowth factors	Migration inhibitory factor (MIF)
Chalones	Maturation factors
Colony-stimulating factor (CSF)	T-cell growth factor [TCGF-interleukin 2 (IL-2)]
Growth factors (transforming growth factor-TSF)	Interleukin 3 (IL-3)
Lymphoctye activation factor [LAF-interleukin 1 (IL-1)]	T-cell replacing factor (TRF)
Lymphotoxin	Thymocyte mitogenic factor (TMF)
Macrophage activation factor (MAF)	Transfer factor
Macrophage chemotactic factor	B-cell growth factor (BCGF)
	Tumor necrosis factor (TNF)
Monoclonal Antibodies	
Monoclonal antibodies to growth-promoting factors	Anti-T-suppressor cell
Anti-T-cell	Antitumor antibody (including antibody fragments and/or conjugates with drugs, toxins, and isotypes)
Antigens	
Tumor-associated antigens	Vaccines
Effector Cells	
Macrophages	T-cell clones
NK cells	T-helper cells
Miscellaneous Approaches	
Allogeneic immunization	Plasmapheresis and ex vivo treatments (activation columns & immunoabsorbents)
Bone marrow transplantation and reconstitution	Virus infection of cells (oncolysates)

techniques could be informative (31). Direct verification of the concept of drug delivery and exposure as a mechanism for resistance can be obtained using certain experimental models. The guinea pig hepatocarcinoma model has already yielded evidence which supports the concept of poor delivery. Active specific immunotherapy increases the vascularity of tumors and prolongs survival times, with an increased cure rate subsequent to the administration of chemotherapy *after* active specific immunotherapy. Studies with monoclonal antibody have indicated that increased vascular permeability has occurred and suggest that the delivery of both drugs and antibody conjugates might be enhanced by such an approach (3).

While the use of biological therapy is at an early stage, it is already apparent that highly purified biologicals can be effective in patients with clinically apparent tumor burdens. Clinical studies with alpha interferon have now demonstrated the responsiveness of drug-resistant lymphoma, melanoma, and renal carcinoma to interferon. These results, along with the early clinical results using monoclonal antibody in clinically apparent disease, confirm the concept that we need not think of biological therapy as a tool that can be used only in patients with undetectable and minimal tumor burdens. While this modality may work best with minimal tumor burdens, a situation which is also true for chemotherapy, biological therapy can be useful as a single modality in clinically apparent disease. It may be even more effective in multimodality treatment regimens for patients with clinically apparent disease. The development of biologicals and biological response modifiers now offers the hope for specific treatment of cancer patients in ways that should significantly enhance the therapeutic/toxic ratio and lessen the problem of nonspecific toxicity, which has been a major impediment to the development of more effective anticancer treatment. The development of biological therapy as the fourth modality of cancer treatment, along with the many recent new leads in tumor biology, makes the future prospects for improving cancer treatment very bright. The 1980s will provide new opportunities to pursue new approaches in cancer treatment. Given these new techniques and new approaches, we must now begin to redesign many of the mechanisms for the discovery and development of new anticancer agents (32). It may well be possible that specifically tailored treatment will require biological therapy to be developed much more individually as compared to the historical development of therapy for broad disease categories. We must be prepared to approach these new horizons and have the flexibility to change and adapt to the challenges and opportunities afforded by biological therapy (32).

REFERENCES

1. DEVITA VT. Progress in cancer management: keynote address. Cancer 51:2401-2409, 1983.
2. ______. The relationship between tumor mass and resistance to chemotherapy: implication for surgical adjuvant treatment of cancer. Cancer 51:1209-1220, 1983.
3. HANNA MG, JR, KEY ME, and OLDHAM RK. The biology of cancer therapy: some insights into adjuvant treatment of metastatic solid tumors. J Biol Resp Modif 2:295-309, 1983.
4. OLDHAM RK, and SMALLEY RV. Immunotherapy: the old and the new. J Biol Resp Modif 2:1-37, 1983.
5. TERRY WD, and ROSENBERG SA. Immunotherapy of Human Cancer. New York, Excerpta Medica, 1982.
6. OLDHAM RK. Biological response modifiers program. J Biol Resp Modif 1:81-100, 1982.
7. ______. Guest editorial: biological response modifiers. JNCI 70:789-796, 1983.
8. HEWITT HB. Animal tumor models and their relevance to human tumor immunology. J Biol Resp Modif 1:107-119, 1982.
9. HERBERMAN RB. Counterpoint: animal tumor models and their relevance to human tumor immunology. J Biol Resp Modif 2:39-46, 1982.
10. SMALLEY RV, and OLDHAM RK. Biological response modifiers: preclinical evaluation and clinical activity. CRC Crit Rev, 1983. In press.
11. SHERWIN SA, KNOST JA, FEIN S, ET AL. A multiple dose phase I trial of recombinant leukocyte A interferon in cancer patients. JAMA 248:2461-2466, 1982.
12. GOLDSTEIN AL, and CHIRIGOS MA. Lymphokines and thymic hormones. *In* Progress in Cancer Research and Therapy. New York, Raven Press, vol 20, 1982.
13. DILLMAN RO, BEAUREGARD JC, MENDELSOHN J, ET AL. Phase I trials of thymosin fraction 5 and thymosin $\alpha 1$. J Biol Resp Modif 1:35-41, 1982.
14. SMALLEY RV, TALMADGE JA, OLDHAM RK, ET AL. The biological response modifiers program: preclinical and clinical studies with thymosin preparations. *In* Thymic Factor Therapy (Byrom NA, and Hobbs JR, eds). London, Academic Press, 1983. In press.
15. ROSENAU W. Lymphotoxin: properties, role and mode of action. Int J Immunopharm 3:1-8, 1981.
16. EVANS CH. Lymphotoxin—an immunologic hormone with anticarcinogenic and antitumor activity. Cancer Immunol Immunother 12:181-190, 1982.
17. WAKSMAN BH. Overview: biology of the lymphokines. *In* Biology of the Lymphokines (Cohen S, Pick E, and Oppenheim JJ, eds). London, Academic Press, 1979, pp 585-616.
18. FOON KA, BERNHARD M, and OLDHAM RK. Monoclonal antibody therapy: assessment by animal tumor models. J Biol Resp Modif 1:277-304, 1982.
19. OLDHAM RK. Monoclonal antibodies in cancer therapy. J Clin Oncol 1:582-590, 1983.
20. FOON, KA, BUNN PA, SCHROFF RW, ET AL. Monoclonal antibody therapy of chronic lymphocytic leukemia and cutaneous T-cell lymphoma: preliminary observations. *In* Monoclonal Antibody and Cancer (Langman RE, Trowbridge IS, and Dulbecco R, eds). New York, Academic Press, 1983. In press.
21. MILLER RA, MALONEY DG, WARNKE R, ET AL. Treatment of B cell lymphoma with monoclonal anti-idiotype antibody. N Engl J Med 306:517-522, 1982.
22. BERNHARD MI, HWANG KM, FOON KA, ET AL. Localization of ^{111}In- and ^{125}I-labeled monoclonal antibody in guinea pigs bearing line 10 hepatocarcinoma tumors. Cancer Res 43:4429-4433, 1983.
23. BERNHARD MI, FOON KA, OELTMANN TN, ET AL. Guinea pig line 10 hepatocarcinoma model: characterization of monoclonal antibody and in vivo effect of unconjugated antibody and antibody conjugated to diphtheria toxin A chain. Cancer Res 43:4420-4428, 1983.
24. GOLDENBERG DM, and DELAND FH. History and status of tumor imaging with radiolabeled antibodies. J Biol Resp Modif 1:121-136, 1982.
25. RITZ J, SALLAN SE, BAST RC, ET AL. Autologous bone-marrow transplantation in CALLA-positive acute lymphoblastic leukemia after in-vitro treatment with J-5 monoclonal antibody and complement. Lancet 2:60-63, 1982.

26. HANNA MG, JR, BRANDHORST JS, and PETERS LC. Active specific immunotherapy of residual micrometastasis: an evaluation of sources, doses and ratios of BCG with tumor cells. Cancer Immunol Immunother 7:165-174, 1979.
27. HANNA MG, JR, and PETERS LC. Morphologic and functional aspects of active specific immunotherapy of established pulmonary metastases in guinea pigs. Cancer Res 41:4001-4009, 1981.
28. HANNA MG, JR, and KEY ME. Immunotherapy of metastases enhances subsequent chemotherapy. Science 217:367-370, 1982.
29. KEY ME, BERNHARD MI, HOYER LC, ET AL. Guinea pig 10 hepatocarcinoma model for monoclonal antibody serotherapy: in vivo localization of a monoclonal antibody in normal and malignant tissues. J Immunol 130:1451-1457, 1983.
30. KEY ME, BRANDHORST JS, and HANNA MG, JR. Synergistic effects of active specific immunotherapy and chemotherapy in guinea pigs with disseminated cancer. J Immunol 130:2987-2992, 1983.
31. GRECO FA, HANDE KR, and FORBES JT. Intracellular localization of antineoplastic agents by immunoperoxidase staining. Proc Am Assoc Cancer Res and ASCO 22:24, 1981.
32. OLDHAM RK. Editorial. Biologicals: new horizons in pharmaceutical development. J Biol Resp Modif 2:199-206, 1983.

Adoptive Immunotherapy of Cancer: Accomplishments and Prospects

Steven A. Rosenberg[1,*]

Adoptive immunotherapy is an approach to cancer treatment that has not received significant evaluation in cancer patients. Recent developments in modern cellular immunology have expanded the opportunities for utilizing adoptive immunotherapy in humans. A variety of animal models have been developed utilizing the adoptive transfer of immune cells that can mediate the regression of established murine tumors. Analyses of these models are defining the important criteria necessary for the application of this approach to humans. Studies of the immune response of human cells to autologous tumors are identifying cells with antitumor reactivity that may be of value in the treatment of human malignancy. Preliminary clinical studies have been performed demonstrating the feasibility of infusing large numbers of activated lymphoid cells into humans. Adoptive immunotherapy is an approach to the treatment of cancer that deserves further study. **[Cancer Treat Rep 68:233–255, 1984]**

Strategies for the immunotherapy of cancer can be divided into active and passive approaches, analogous to the use of these terms in the treatment of infectious diseases. Active immunotherapy refers to the immunization of the tumor-bearing host with materials designed to elicit an immune reaction capable of eliminating or retarding tumor growth. Active immunotherapy can be either specific (immunization with specific antigens present on the tumor) or nonspecific (administration of general immune stimulators). Most attempts at the immunotherapy of cancer in humans in the last several decades have involved active immunotherapy utilizing nonspecific immune stimulators such as BCG or *Corynebacterium parvum*, with the hope that a nonspecific increase in immune reactivity will concomitantly result in an augmentation of the putative antitumor immunologic response of the tumor-bearing host. The very large number of clinical trials of both specific and nonspecific active immunotherapy of human cancer performed in the last decade were almost universally unsuccessful and enthusiasm for this approach to immunotherapy has waned considerably.

The second category of immunotherapy, passive immunotherapy, involves the transfer to the tumor-bearing host of previously sensitized immunologic reagents, such as cells or antibody, that have the ability to mediate antitumor responses. The term "adoptive immunotherapy" is usually used to connote passive immunotherapy with cells such as lymphocytes or macrophages. Passive approaches to immunotherapy have been rarely utilized in the therapy of human cancer. Early studies attempting to use either serum or cell transfer for cancer treatment preceded much of the recent explosion of information in modern immunology (1). The development of methods for generating monoclonal antibodies has provided great impetus to the study of passive immunotherapy using specific antitumor antibodies raised by immunizing mice with human tumors. Multiple trials are now in progress attempting to use these monoclonal antibodies for both tumor localization and therapy.

Passive immunotherapy with sensitized cells, referred to as "adoptive immunotherapy" throughout this paper, has received very little attention in humans because of the theoretic and technical difficulties that are associated with performing these clinical trials. Adoptive immunotherapy, however, has substantial theoretic attractiveness as an approach to treating human cancer. Soon after the immunologic basis of tissue rejection was understood, serum and cell transfer experiments clearly identified the cellular arm of the immune response as crucial in mediating the rejection of allogeneic grafts and syngeneic tumor in animal studies. In most experimental systems, the transfer of a specific antibody is not capable of transferring immunity against cellular antigens, whereas the transfer of immune lymphocytes is capable of transferring immunity. Adoptive immunotherapy with cells of

---

[1] Surgery Branch, National Cancer Institute, Bethesda, MD.

[*] *Reprint requests to:* Steven A. Rosenberg, MD, Surgery Branch, National Cancer Institute, Bldg 10, Rm 10N116, National Institutes of Health, Bethesda, MD 20205.

appropriate reactivity should be highly specific, with minimal toxicity to normal tissues. Because most cancer patients are significantly immunosuppressed, any approach to immunotherapy that depends on high levels of immune competence of the host are unlikely to succeed. This is probably a major factor that led to the failure of attempts utilizing active immunotherapeutic approaches. Because adoptive immunotherapy involves the transfer of immunocompetent cells to the tumor-bearing host, there is less need for full immunocompetence of the host than is needed for other therapeutic approaches. Also, because of the specificity of adoptive immunotherapy, these treatments are not likely to be immunosuppressive. For this reason, too, adoptive immunotherapy is an approach that would seem ideally suited for combination with radiation therapy and chemotherapy, which themselves may be immunosuppressive.

In attempting to construct reasonable strategies for the adoptive immunotherapy of human cancer, it is helpful to consider successful animal models of adoptive immunotherapy in an attempt to identify the essential features required for the success of this therapeutic approach (1-3).

Four major principles have evolved from animal studies that must be considered in the development of the adoptive immunotherapy of cancer in humans (table 1, left-hand column; refs 1-12): (*a*) In most animal models, the adoptive transfer of syngeneic cells is far more effective than the transfer of allogeneic or xenogeneic cells. In many models, only syngeneic cells are effective in mediating tumor regression (4-6); (*b*) The adoptive transfer of very large numbers of sensitized cells is necessary. Although the number of cells transferred will be governed by their level of activity and traffic patterns when reinjected in vivo, in general, about 10^8 highly sensitized fresh lymphocytes are required to mediate the regression of tumor nodules < 1 cm in most murine models (4-12); (*c*) The use of cells from highly immunized syngeneic animals has been essential to the success of adoptive immunotherapy. In some experimental protocols, months of repetitive immunizations with tumor cells have been required to obtain cells with sufficient antitumor reactivity to mediate antitumor effects (6,7); (*d*) Recent work has pointed to the importance of suppressor mechanisms in tumor-bearing individuals. In a variety of animal models, successful therapeutic results require the elimination of host suppressor cells, prior to the adoptive transfer of cells with antitumor activity (8).

TABLE 1.—Requirements for successful adoptive immunotherapy

Murine models	Possible approach in humans
Syngeneic cells are essential	Use autologous cells
Large Nos. of cells are necessary (transfer of pooled cells from many animals)	Expand cells in IL-2 (or by repetitive restimulation)
Cells obtained from hyperimmunized animals	Use cloned cells from tumor-bearing host (? after in vitro sensitization)
Eliminate suppressor cells with cyclophosphamide or irradiation	Same

Until recently, it has not been possible to apply many of the principles learned from experimental models to the adoptive immunotherapy of human cancer. Most humans do not have syngeneic counterparts available for cell donation. Similarly, it is not feasible to hyperimmunize humans against cancer antigens, and only limited cell numbers can be harvested from human donors.

Recent developments in cellular immunology have provided a means, at least theoretically, for developing an approach to adoptive immunotherapy that complies with each of the principles thought to be important in animal models. This approach to adoptive immunotherapy takes advantage of the recent discovery of Interleukin-2 (IL-2 or T-cell growth factor) to expand in vitro T-lymphoid cells with maintenance of functional specificity (13). The approach we have developed is outlined in table 1 (right-hand column).

IL-2 is a lymphokine, produced by a subpopulation of lymphoid cells, that can mediate the growth of T-lymphoid cells in vitro. Using IL-2, it is also possible to clone lymphoid cells of the major T-cell subpopulations (14,15). Thus, it is possible to expand in vitro T-lymphoid cells from tumor-bearing individuals. Because T cells can grow in vitro with a doubling time of < 48 hours, > 2 × 10^{13} cells can be generated within 2 months, beginning with only 10^4 starting cells (16).

The requirement for highly sensitized cells with antitumor reactivity can also theoretically be met in humans using these techniques. If cells with antitumor reactivity exist or can be generated from the lymphoid cells of the tumor-bearing host, these reactive cells can potentially be cloned in vitro and large populations can be derived from single starting cells. It would thus be possible to generate an idealized hyperimmune lymphoid population, ie, a population in which every lymphoid cell had the desired reactivity. Finally, effective techniques for eliminating suppressor cells in experimental animals, using either radiation or cyclophosphamide, are potentially applicable to the human, though it is not yet clear that these treatments eliminate the suppressor cell subclass in the human that is capable of inhibiting the function of adoptively transferred lymphocytes.

This report will describe our efforts to develop an immunotherapeutic approach to the treatment of cancer based on the ability to identify, isolate, clone, expand, and infuse into tumor-bearing humans autologous, sensitized lymphocytes with antitumor reactivity. This approach is in the early stages of development and many unsolved problems remain.

The Adoptive Transfer of Immune Lymphocytes Can Lead to Regression of Established Tumors

Several tumor models have been developed in the mouse, rat, and guinea pig that demonstrate that the adoptive transfer of fresh, sensitized syngeneic lymphocytes can mediate the regression of established transplantable tumors (1-12). In most of these models, the tumor is implanted and allowed to grow for 4-7 days to form a palpable nodule prior to instituting therapy with immune lymphocytes. We have primarily studied two tumor models, the FBL-3 lymphoma in C57BL/6 mice and the Meth A sarcoma in BALB/c mice.

The Meth A fibrosarcoma is a methylcholanthrene-induced sarcoma that grows progressively in normal BALB/c or (BALB/c × C57BL/6)F_1 mice and is passaged in ascitic form. To obtain cells with anti-Meth A tumor activity, 10^7 Meth A tumor cells are admixed with 100 μg of killed *C. parvum* bacteria and injected into mice (8). Following injection, this tumor grows for about 10 days and then completely regresses. The splenocytes from these mice are obtained 4-6 weeks later and used for adoptive transfer.

In a characteristic experiment, BALB/c mice or (BALB/c × C57BL/6)F_1 mice were given 500 rads of whole body irradiation and 2-4 hours later, 10^6 Meth A cells were injected intradermally. Four days later, when the tumor had grown to a 1-2-mm nodule, 10^8 immune splenocytes or control splenocytes were injected iv into different groups of mice. Only a single cell treatment was given. Both tumor size and survival were monitored. A characteristic experiment is shown in figures 1, 2, and 3. In mice injected with control splenocytes, the tumor grew invariably and resulted in death in approximately 25-30 days (fig 1). Following injection of immune cells, however, the tumor grew for about 10 days and then slowly regressed completely. Sequential photographs of a single mouse treated with either normal lymphocytes or immune lymphocytes are shown in figures 2 and 3. In the mouse that received the adoptive transfer of normal lymphocytes, the Meth A tumor continued to grow progressively and, for about 10 days, tumor growth was approximately the same in mice receiving normal and immune lymphocytes. At about the 8th to 11th day, however, the central area of ischemic necrosis that occurs in most of the tumors became somewhat larger in the mouse that received immune splenocytes. Beginning on Day 13 after tumor injection (9 days after lymphocyte injection), a marked difference appeared between the tumors. In mice that had received immune splenocytes, the tumor ceased to grow and the central area of necrosis expanded within the tumor. By Days 20-25, the tumor had completely disappeared in animals receiving immune splenocytes, whereas it continued to grow in animals that received normal splenocytes. The pattern of regression seen in figures 2 and 3 is characteristic.

We have also studied the ability of adoptively transferred lymphocytes to treat a Friend virus-induced lymphoma-leukemia called the FBL-3 tumor (11,12). This tumor grows and regresses in about 70% of normal C57BL/6 mice if injected im, although it grows progressively in all mice that receive 500 rads of total body irradiation. To immunize cells against the FBL-3 lymphoma, mice receive an im injection of 10^7 live FBL-3 cells. Tumors regress after approximately 2 weeks in about 70% of mice, and these mice are used as spleen donors for adoptive immunotherapy approximately 2 weeks after complete regression has taken place. In this treatment model, C57BL/6 mice were given 500 rads of total whole body irradiation and 2-4 hours later, 10^7 live FBL-3 cells were injected either intradermally or into the right-hand footpad. By 4-5 days later, tumor was easily palpable and was disseminated throughout the regional lymph nodes and peripheral circulation. Five days after tumor injection, mice were randomly assigned to groups and injected iv with either sensitized or control lymphocytes. A characteristic experiment is shown in figure 4 (12). Note that the adoptive transfer of 10^8 immune splenocytes caused complete regression of the tumor with survival in 80% of the mice. Adoptive transfer of 5 × 10^7 immune splenocytes delayed tumor growth and improved survival, but was not capable of curing mice. The injection of 10^8 nonimmune lymphocytes had no effect on tumor growth.

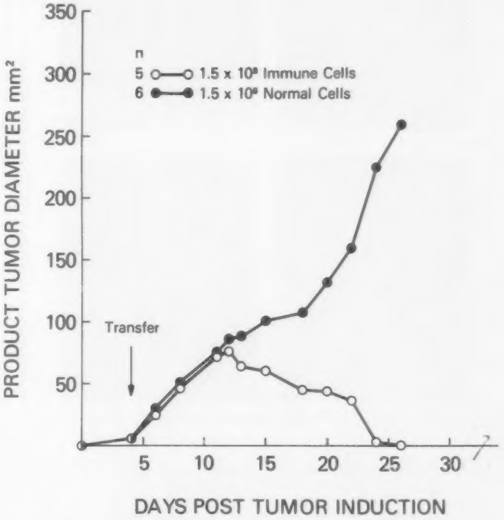


FIGURE 1.—(C57BL/6 × BALB/c)F_1 mice were irradiated with 500 rads and injected with 10^6 Meth A sarcoma cells intradermally. On Day 4, they received the adoptive transfer of a single infusion of normal or immune lymphocytes. Mice receiving normal lymphocytes exhibited progressive growth of tumor and died. Animals receiving immune lymphocytes ultimately underwent complete regression of their tumor and were long-term survivors.

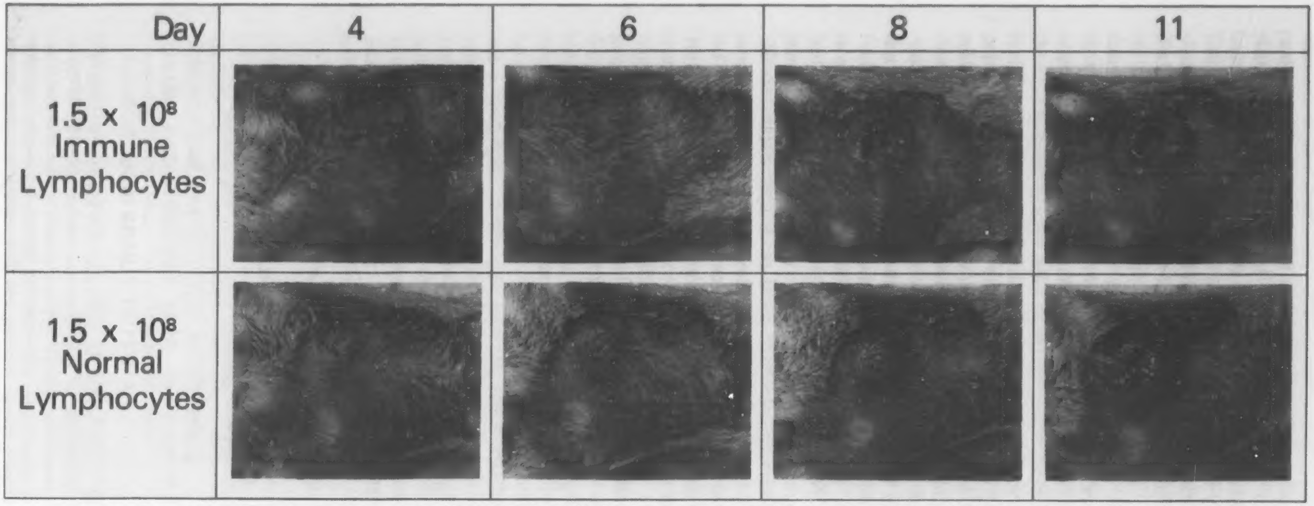

Day
4
6
8
11
1.5 x 10^8 Immune Lymphocytes
1.5 x 10^8 Normal Lymphocytes

FIGURE 2.—Sequential photographs of a single mouse with an intradermal MCA sarcoma. The top panel shows a mouse that received immune lymphocytes 4 days after intradermal inoculation of tumor. The lower panel shows a mouse that received normal lymphocyte at the same time. For the first 11 days, tumor growth is approximately equal in both mice.

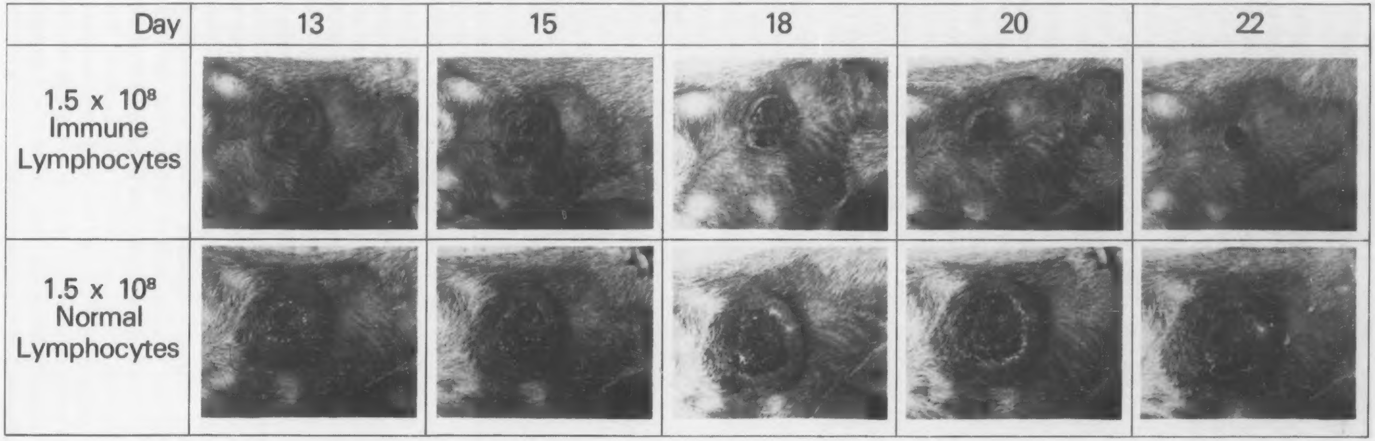

Day
13
15
18
20
22
1.5 x 10^8 Immune Lymphocytes
1.5 x 10^8 Normal Lymphocytes

FIGURE 3.—Continuation of sequential photographs of the mice shown in fig 2. Note that the mice receiving immune lymphocytes undergo complete regression of their tumor. The tumor grows progressively in the mouse that received normal lymphocytes. Note that a central area of ischemic necrosis exists in the tumor in both mice. However, an expanding viable rim of tumor exists in mice that did not receive immune lymphocytes.

Thus, these two tumor models demonstrate several important principles of adoptive immunotherapy. The adoptive transfer of immune cells can mediate the regression of established tumors. Prior to cell transfer, mice were immunosuppressed to eliminate suppressor factors. Tumors grew for approximately 10 days after lymphocyte injection and then regressed completely. These two murine tumor systems have been used to study the principles and mechanisms that govern successful adoptive immunotherapy.

Which Lymphocyte Subpopulation Mediates the Adoptive Immunotherapy of Tumors?

A major challenge confronting attempts to successfully use adoptive immunotherapy for the treatment of human tumors involves the identification of the appropriate cell types necessary for use in adoptive transfer. An understanding of the lymphocyte subpopulations that mediate tumor regression in murine models would aid in attempts to search for corresponding cells in the human. There is much controversy concerning this point (17). Using a skin allograft as well as an allogeneic tumor model, Loveland et al (18,19) implicated Lyt-1$^+$2$^-$ cells as the important cell type involved in mediating the regression of allogeneic tissue. Others (20,21) have implicated an Lyt-2$^+$ cytotoxic cell. In our experiments, we selectively depleted immune splenocytes of either Lyt-1$^+$ or Lyt-2$^+$ cells, using monoclonal antibody and complement prior to adoptively transferring these cells into mice bearing the FBL-3 or the Meth A tumor. The cumulative results of two experiments in the FBL-3 tumor system and three experiments in the Meth A tumor system are shown in table 2 (17). The adoptive transfer of splenocytes treated with complement alone in either the FBL-3 or Meth A tumor system was capable of curing 95% and 65% of mice, respectively. In both the FBL-3 and Meth A tumor systems, treatment with either anti-Lyt-1 or Lyt-2 sera plus complement abrogated the ability of these cells to mediate the adoptive rejection of the established tumors. However, different results were obtained in these two major models when the ability of mixtures of these cells to cure tumors was studied. In the FBL-3 tumor, 100% of mice were cured by mixing the cell populations that had previously been depleted of Lyt-1$^+$ cells with cell populations that had been depleted of Lyt-2$^+$ cells. This result implied that two different cell types were involved in mediating the regression of the FBL-3 tumor in vivo and that one cell type bears the Lyt-1$^+$ marker and the second bears the Lyt-2$^+$ marker. In the treatment of the Meth A tumor, however, the mixture of Lyt-1- and Lyt-2-depleted cells was not capable of curing mice. It appeared in this tumor model that a cell type bearing both the Lyt-1 and Lyt-2 phenotypic marker was essential for mediating tumor regression.

It thus appeared that the tumor model system utilized was responsible for determining the cells required for successful treatment of each tumor. Because these ex-

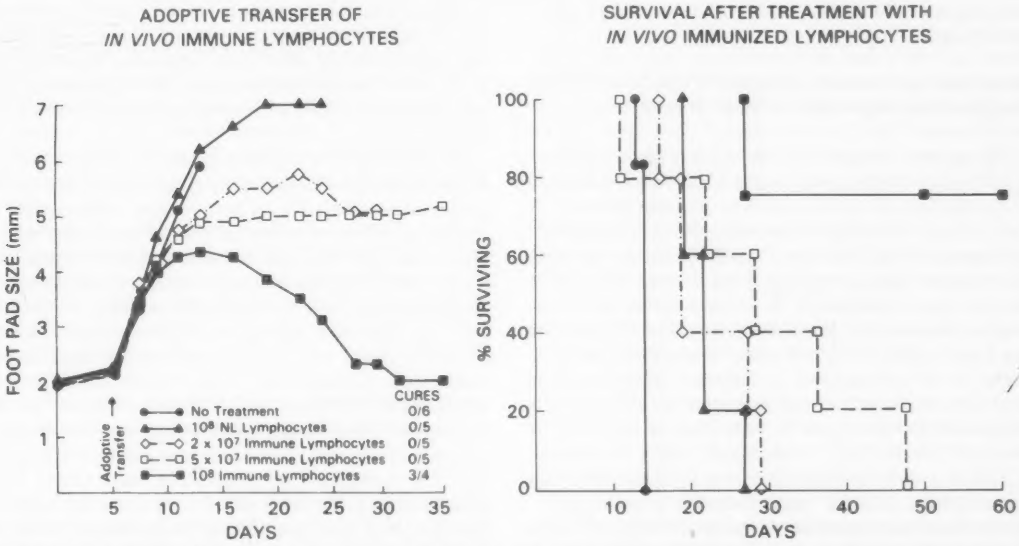


FIGURE 4.—Mice with disseminated solid FBL-3 lymphoma were treated with in vivo immunized lymphocytes and normal lymphocytes. The footpad tumor size is shown on the left and survival is shown on the right. Treatment with 10^8 immune cells caused necrosis of tumors and cured mice. 10^8 normal lymphocytes or lower Nos. of immune lymphocytes could not mediate regression of FBL-3 lymphoma or cure mice with this tumor (ref 12).

TABLE 2.—Lyt phenotype of lymphoid subpopulations that mediate adoptive rejection of the FBL-3 and Meth A syngeneic tumors (*from* ref 17)

Treatment of immune cells*	FBL-3 tumor	Meth A tumor
	No. cured/ total No. (%)	No. treated/ total No. (%)
None	2/4 (50)	11/18 (61)
Complement (C)	19/20 (95)	12/20 (65)
Anti-Lyt-1 + C	0/11 (0)	0/18 (0)
Anti-Lyt-2 + C	2/16 (13)	0/19 (0)
Group 3 + Group 4†	11/11 (100)	0/13 (0)

* All mice were treated iv with 10^8 viable cells.

†Mice received 5 × 10^7 cells from Group 3 and 5 × 10^7 cells from Group 4.

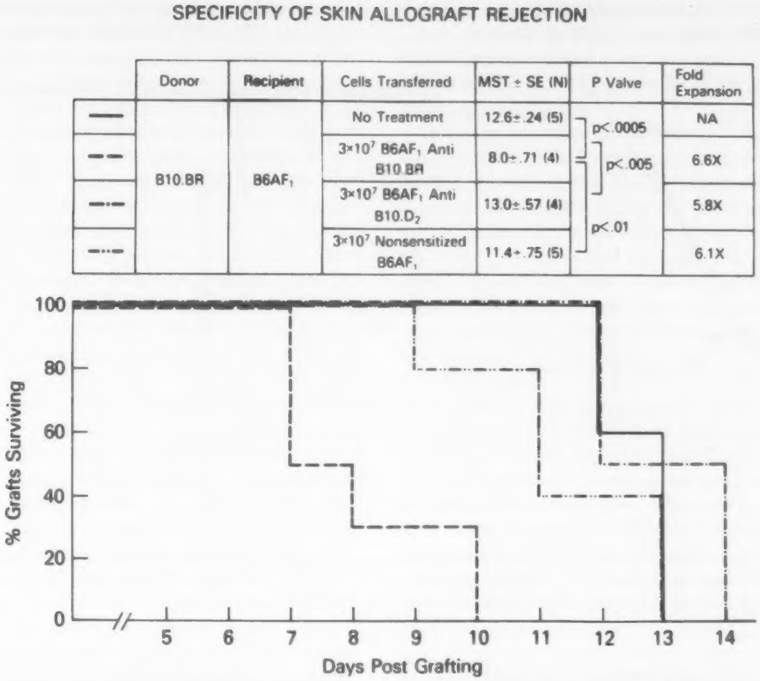
periments were done in different animal strains, the possibility existed that differential susceptibility of splenocytes of these strains to the monoclonal antibodies might influence the result. We thus repeated these experiments, utilizing (C57BL/6 × BALB/c)F_1 hybrid mice (17). In these experiments, the FBL-3 and Meth A tumors were grown in these hybrid mice, and then similar depletion experiments were performed to determine which cell was responsible for mediating tumor regression. Again in this tumor system, the FBL-3 tumor was cured by adoptive transfer of a mixture of both anti-Lyt-1 plus anti-Lyt-2 depleted cells, whereas each single depleted population alone was incapable of curing mice. In the Meth A tumor system, however, neither of these single depletions nor a mixture of the two depleted cell populations was capable of curing mice. It thus appears that different tumors may be affected by the adoptive transfer of differing cell types and that multiple cell subpopulations may be involved in mediating the regression of an established tumor.

Expanded and Cloned Lymphoid Cells Can Mediate the Adoptive Regression of Skin Allografts

Our projected approach to the adoptive immunotherapy of human tumors involves the expansion and cloning of lymphoid cells in IL-2 prior to adoptive transfer. It thus became necessary to determine whether lymphoid cells expanded in IL-2 were capable of mediating adoptive rejection when systemically transferred. Cell traffic studies that we performed in both the mouse and in the human demonstrated that these cells tended to "home" to the lung in the first hours after adoptive transfer, followed by an accumulation in liver and spleen over the next several days (22). A well-characterized skin allograft rejection model developed by Sugarbaker and Chang (23) was utilized in these experiments. Mice used were (C57BL/6 × A)F_1 mice that underwent skin grafting with ear skin from B10.BR mice. This skin is normally rejected in untreated animals in approximately 12-14 days. The iv adoptive transfer of appropriately sensitized fresh splenocytes can accelerate skin graft rejection to approximately 8 days. This system served as a useful model to determine whether appropriately sensitized lymphoid cells expanded in IL-2 were capable of mediating in vivo effects. This in vivo test measured not only the immunologic capacity of these cells, but their ability to traffic to areas appropriate to elicit the desired immune response.

A variety of sensitized and nonsensitized cells were expanded in IL-2 prior to adoptive transfer in this model (24). A characteristic experiment is shown in figure 5. B6AF_1 recipients of B10.BR donor skin that received no cell transfer exhibited a skin graft survival time of 12.6 ± 0.2 days. When B6AF_1 cells sensitized to B10.BR lymphocytes in in vitro sensitization cultures were expanded in IL-2 and adoptively transferred, they were capable of mediating the accelerated rejection of B10.BR skin in 8.0 ± 0.7 days ($P < 0.0005$, compared to untreated mice). Only these specifically sensitized and expanded cells were capable of mediating accelerated rejection of B10.BR skin. B6AF_1 anti-B10.D_2 cells or nonsensitized B6AF_1 cells that had been simultaneously expanded in IL-2 and adoptively transferred were not capable of accelerating skin graft rejection. These experiments were the first to show that cells expanded in IL-2 were capable of mediating tissue rejection following iv injection (24).

This skin allograft rejection model was further utilized to study whether cloned lymphocyte populations with appropriate reactivity for target antigens were capable of mediating adoptive rejection (25). These experiments were conducted both in the accelerated skin allograft rejection model described above, as well as by studying the ability of cloned cells to mediate the rejection of skin allografts in C57BL/6 nude mice. Highly cytolytic nonproliferative clones and highly proliferative noncytolytic clones were isolated from C57BL/6 anti-DBA/2 in vitro sensitization cultures, using limiting dilution cloning techniques. These clones were subcloned several times to assure that the population arose from a single progenitor cell. The techniques for generating these clones have been described elsewhere (26). Identical results were obtained in both the skin allograft acceleration model, as well as in the rejection of skin by nude mice. Highly cytolytic clones were incapable of mediating skin allograft rejection. However, clones with in vitro proliferative and



	Donor	Recipient	Cells Transferred	MST ± SE (N)	P Valve	Fold Expansion
—	B10.BR	B6AF$_1$	No Treatment	12.6±.24 (5)	p<.0005	NA
– –			3×10^7 B6AF$_1$ Anti B10.BR	8.0±.71 (4)	p<.005	6.6X
–·–			3×10^7 B6AF$_1$ Anti B10.D$_2$	13.0±.57 (4)	p<.01	5.8X
–··–			3×10^7 Nonsensitized B6AF$_1$	11.4±.75 (5)		6.1X

FIGURE 5.—Skin allograft rejection following the adoptive transfer of nonsensitized B6AF$_1$ lymphocytes or B6AF$_1$ lymphocytes sensitized to the relevant B10.BR or the irrelevant B10.D$_2$ target antigens. All lymphocytes in this experiment were expanded in IL-2 prior to iv injection. Note that only the lymphocytes sensitized to the B10.BR target are capable of accelerating rejection of this skin graft (ref 24).

no cytolytic activity were highly effective in both accelerating skin graft rejection and in mediating rejection of allogeneic skin in nude mice. Two separate proliferative clones, one with specificity directed against I-A antigens and the second against MLL or a closely linked antigen, were both highly effective in mediating skin rejection (25).

These experiments clearly indicated that the reinjection of cells with purely cytolytic activity was incapable of mediating graft rejection, whereas proliferative cells were highly effective. We then attempted to extend these studies to a model of the adoptive rejection of an established syngeneic tumor.

Expanded Cell Lines and Clones Are Effective in the Adoptive Immunotherapy of a Syngeneic Lymphoma

The FBL-3 lymphoma model described earlier was used to test the ability of expanded and cloned cells to impact on the growth of this tumor in vivo. Immune splenocytes were boosted by in vitro sensitization and then expanded in IL-2 prior to iv injection into tumor-bearing mice (11,12,27). Mice were treated on Day 4 or 5 of tumor growth at a time when both local tumor as well as disseminated tumor in lymph nodes and peripheral blood were present (12). A characteristic experiment is shown in figure 6. As seen on the left, tumors grew rapidly in mice that received either no therapy or therapy with 5 × 10^7 normal splenocytes expanded in IL-2. However, both 2.5 and 5 × 10^7 immune splenocytes expanded in IL-2 were capable of causing regression of the lymphoma (fig 6, left). All mice in both of the control groups died in 15–25 days, whereas 11 of 12 mice treated with expanded cells were permanently cured (fig 6, right). These experiments demonstrated that the adoptive transfer of sensitized cells expanded in IL-2 was capable of curing mice with a disseminated lymphoma. These cells appeared to be more efficient than fresh splenocytes, since at least 10^8 fresh splenocytes were required to mediate cures seen with 2.5 × 10^7 expanded cells.

The FBL-3 lymphoma model was also utilized to help determine which lymphocyte subpopulations expanded in IL-2 were the effective cells in mediating tumor regression (28). A replica-plate screening technique was developed that enables the screening of > 1000 clones/day

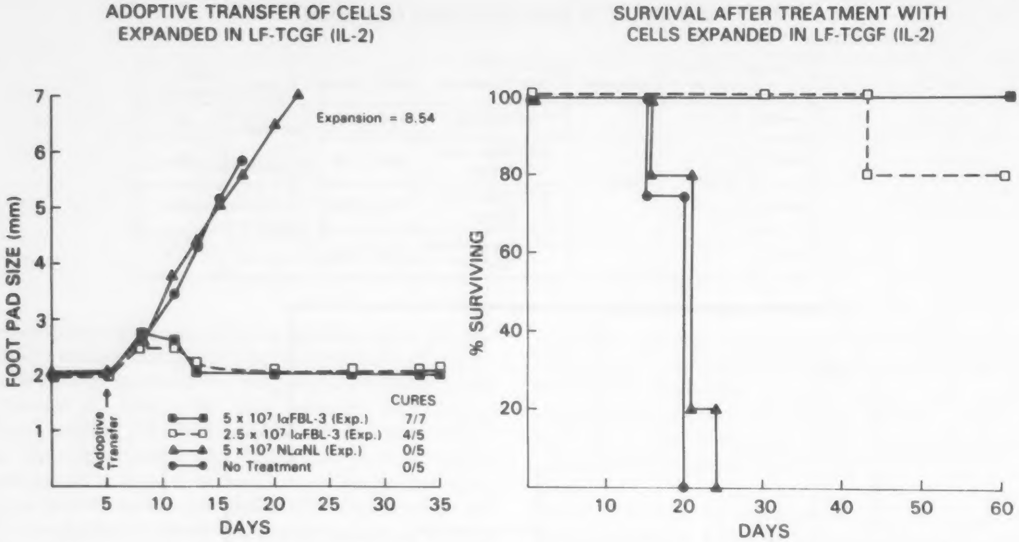


FIGURE 6.—Immune cells that were sensitized to the FBL-3 lymphoma and expanded in IL-2 were used to treat mice with the solid disseminated FBL-3 lymphoma. 2.5 and 5 × 10^7 immune cells were capable of mediating regression of lymphoma and curing mice. Normal lymphocytes expanded in IL-2 had no impact on tumor growth or survival (ref 12).

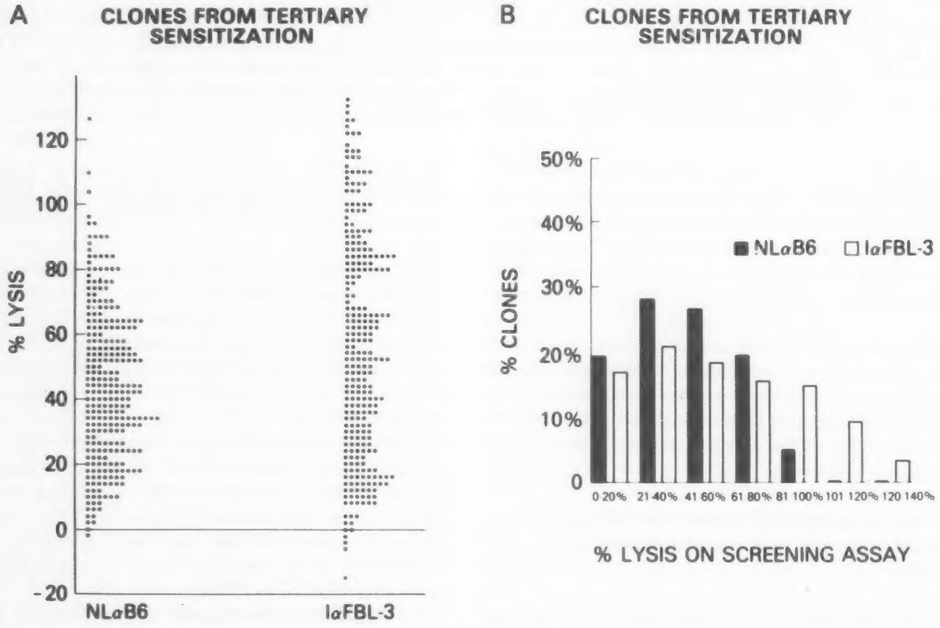


FIGURE 7.—A replica plate screening assay was used to identify cloned lymphoid populations with lytic activity to the FBL-3 lymphoma. The results of a single assay are shown on the left. Each dot represents a separate clone. The same data shown as a % of the total number of clones are shown on the right. Note that a higher percentage of highly cytotoxic clones was found in immune cultures, as compared to control cultures (ref 27).

to identify clones with either cytolytic or proliferative activity. This technique has been described elsewhere (27). In brief, splenocytes following in vitro sensitization are plated by limited dilution techniques into microtiter wells. Plates that have between 30% and 50% of wells with growth are assayed by removing one-half of the contents of each well into a replica plate containing the appropriate target cells. These transferred cells are then tested for their lytic ability or for the ability of target cells to stimulate proliferation. At the completion of the assay, wells with interesting activity are identified and these wells are harvested from the original plate for growth and recloning. A typical assay is presented in figure 7. Each dot on the left side of figure 7 shows a clone from limiting dilution plates screened for the ability to lyse the FBL-3 tumor (27). Cells were also cloned and tested from a control culture. Though there was a wide variability of clones capable of lysing the FBL-3 tumor, there was a higher percentage of highly lytic clones in the immune cultures compared to the control cultures. Highly lytic clones were harvested, expanded in IL-2, and tested (fig 8). Clones 2, 17, and 19 were highly lytic clones with anti-FBL-3 lytic activity. Clone 9′ was a control clone that had no lytic activity. The former three clones, but not the latter clone, maximally lysed the FBL-3 target at effector to target ratios of 0.1:1. Even at ratios of 10:1, clone 9′ was not lytic for the FBL-3 tumor. None of these clones were lytic for a control syngeneic, except for a non-cross-reacting C57BL/6 sarcoma called MCA-103.

Utilizing this technique, clones with a high degree of proliferative activity to the FBL-3 tumor were also isolated. The proliferative and cytolytic specificity of one such clone (clone 28-7) is shown in table 3 (28). This clone had a high degree of proliferative specificity for the FBL-3 tumor and did not proliferate when stimulated with other syngeneic or allogeneic tumors. This particular recloned population had a very low level of lysis against the FBL-3 tumor, exhibiting only 29 ± 2% lysis at effector to target ratios of 100:1.

These proliferative and cytolytic clones were used to determine which cell subpopulations were capable of affecting growth of the FBL-3 tumor when these cloned lines were injected systemically (28). The results of several experiments are presented in table 4. As can be seen, the cytolytic clones had no impact in mice with established FBL-3 lymphomas. However, in four of five experiments, proliferative cell lines and proliferative clones were capable of significantly prolonging survival of mice bearing the FBL-3 lymphoma.

Several points were of interest in these experiments. As was seen in the skin allograft model, cytolytic clones were ineffective in mediating tissue rejection. The proliferative lines and clones in the FBL-3 model system, although capable of prolonging survival, were not as effective as the whole cell lines expanded in IL-2. Mixed cell lines were capable of curing the majority of mice (fig 6), whereas cures with the proliferative clones alone were only rarely seen. These findings corroborated our findings in the skin allograft system, which points to the importance of proliferative cells in mediating tissue rejection. It does not appear, however, that an expanded proliferative cell line alone is fully effective in mediating tumor rejection. The participation of other cell types missing in individual cloned lines appears to be necessary to cure mice of the FBL-3 lymphoma. Studies presented in a previous section indicated that at least two cell subpopulations were necessary for successful treatment of mice with the FBL-3 tumor, and perhaps one of these cell types was missing in these cloned lines. It appears, how-

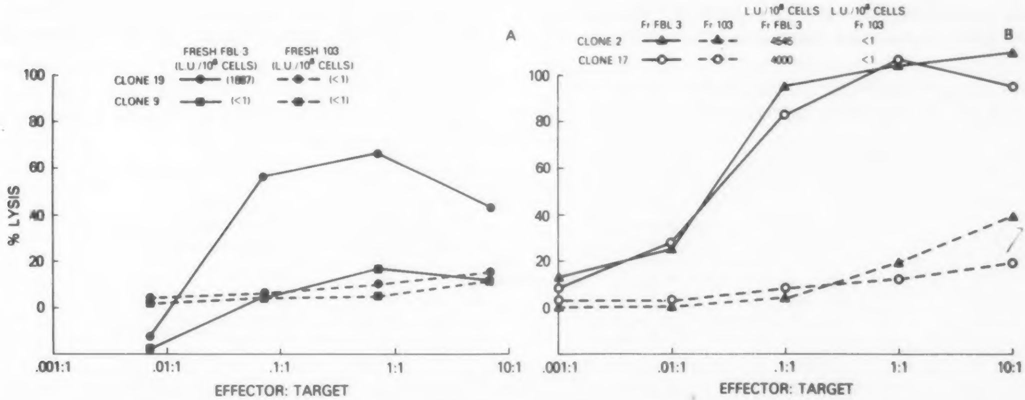


FIGURE 8.—Four clones (clones 2, 17, and 19 were cytolytic and clone 9′ was noncytolytic) were isolated from a replica plate screening assay and expanded in IL-2 for 2½ mos. The 3 cytolytic clones maintained high degrees of lytic activity against the FBL-3 tumor and exhibited no lysis of the non-cross-reactive syngeneic methylcholanthrene-induced sarcoma 103. Clone 9′ was not lytic for either the FBL-3 or the 103 tumor (ref 27).

TABLE 3.—Proliferative and cytotoxic specificity of clone 28-7 (*from* ref 28)

Stimulator	SI†	Target	% lysis ± SEM* Effector:target ratio 100:1	10:1	1:1
None	—				
C57BL/6 lymphocytes	0.4	C57BL/6 lymphocytes	-20 ± 4	-17 ± 2	-15 ± 2
FBL-3	16.3‡	FBL-3	29 ± 2	25 ± 2	6 ± 1
EL-4	0.04	EL-4	80 ± 2	81 ± 5	39 ± 1
P815	0.3	P815	10 ± 1	7 ± 1	2 ± 1
53	0.3	53	-13 ± 8	-23 ± 4	-31 ± 9
Meth A	0.4	Meth A	3 ± 1	1 ± 1	0 ± 1

* 4-hr chromium release assay.

$$\dagger SI = \frac{\text{(cpm clone + stimulator)}}{\text{(cpm clone alone) + (cpm stimulator alone)}}.$$

‡Clone 28-7 alone = 715 cpm, FBL-3 alone = 4139 cpm, clone + FBL-3 = 79,170 cpm; 48-hr proliferative assay.

ever, that cells with proliferative function have some role in mediating the rejection of this syngeneic tumor.

The Systemic Administration of IL-2 Can Enhance the Ability of Expanded Adoptively Transferred Cells to Cure Mice With the FBL-3 Lymphoma

Because cells expanded in IL-2 are dependent on the continuous presence of IL-2 for their long-term survival, we have explored the possibility that the systemic administration of IL-2 may enhance the therapeutic effectiveness of adoptively transferred cells expanded in IL-2. The FBL-3 lymphoma model described previously was utilized in these studies.

In preliminary experiments, we studied the fate of IL-2 following either iv, ip, or sc injection into C57BL/6 mice (fig 9). Following iv administration, the serum half-life of IL-2 was 3.7 ± 0.8 minutes (29). However, sustained serum levels could be achieved if IL-2 was administered either ip or sc (29).

To evaluate the ability of IL-2 to enhance the ability of adoptively transferred expanded lymphocytes to cure mice with the FBL-3 lymphoma, we treated tumor-bearing mice with small numbers of expanded lymphocytes in conjunction with the ip administration of IL-2, as shown in table 5. In the experiment shown in table 5, mice bearing the FBL-3 tumor were treated with sensitized lymphocytes expanded in IL-2, and they also received IL-2 ip for 3 days after cell administration. The use of IL-2 plus expanded lymphocytes resulted in an improved survival rate when compared to the use of expanded lymphocytes alone. This result has thus far been seen in six of seven experiments utilizing both a murine IL-2 (partially purified from an EL-4 thymoma line) or a human-derived IL-2 (homogeneous IL-2 from the Jurkat cell line, provided by Dr. Richard Robb, Dupont Co). This approach to enhancing the effect of adoptively transferred cells warrants further evaluation.

Activated Killer Cells May Be Effective for Use in the Adoptive Immunotherapy of Tumors

It has generally been assumed that the most effective cells for adoptive immunotherapy would be those with

TABLE 4.—Survival after adoptive transfer of cytotoxic clones and proliferative lines and clones (*from* ref 28)

Experiment No.	Survival (MST* ± SEM) No treatment (n)	Treatment (n)	*P* value†
	Cytotoxic clones		
A	16.5 ± 0.9 (6)	17.8 ± 1.0 (5)	Not significant
B	20.4 ± 1.1 (8)	19.6 ± 2.9 (5)	Not significant
C	17.8 ± 1.8 (5)	19.2 ± 0.5 (5)	Not significant
	Proliferative lines		
A	19.9 ± 0.4 (8)	24 ± 2 (5)	< 0.05
B	14.7 ± 1.7 (6)	28.2 ± 10.2 (6)	Not significant
C	16.7 ± 0.4 (6)	22.4 ± 0.6 (5)	< 0.001
	Proliferative clones		
D	16.3 ± 1.2 (6)	25.8 ± 2.4 (5)	< 0.01
E	19.4 ± 0.4 (7)	27.3 ± 2.0	< 0.01

* MST = mean survival time.

†Clone or line vs no treatment.

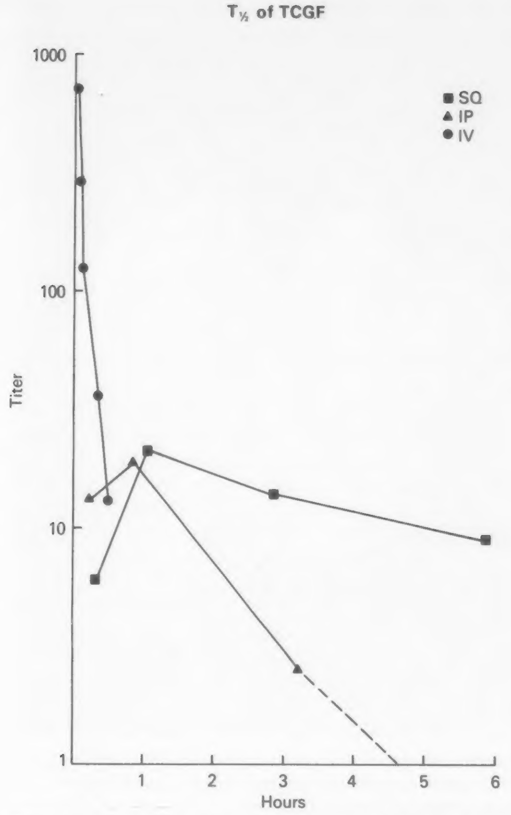


FIGURE 9.—The serum half-life of IL-2 was measured following iv injection into mice. Following iv injection, the serum half-life of IL-2 activity was 3.7 ± 0.8 mins (mean ± 1 SEM of nine experiments). More sustained levels in the serum could be achieved by injecting IL-2 either ip or sc (ref 29).

specific reactivity to the putative tumor antigens present on cancer cells. The studies previously described in this report have dealt with cells with specific reactivity to unique target antigens on syngeneic tumors.

Because of the very poor (if present at all) immunogenicity of human tumors, the incidence of immune lymphocytes specifically reactive against these tumor antigens will be very low and these cells will be difficult to identify and isolate. We have thus explored the ability of using nonspecifically activated cell with antitumor reactivity in the adoptive immunotherapy of murine tumors. We have shown that a variety of techniques for activating either murine or human cells are capable of generating cells with lytic activity for fresh autologous tumor cells (30-36). Activation of normal lymphocytes with either IL-2, lectins, or by stimulation with normal allogeneic cells generates cells which have been collectively referred to as activated killer (AK) cells. These AK cells are capable of lysing (in short-term chromium-release assays) fresh syngeneic tumor cells that are resistant to lysis by natural killer (NK) cells. An example of the ability of IL-2 to generate AK cells is shown in table 6 (36). In this experiment, splenocytes from C57BL/6 mice were tested against a variety of target cells for their ability to lyse tumor and normal syngeneic cells. The lytic activity of the splenocytes was tested before and after incubation in medium containing IL-2. Fresh uncultured splenocytes were capable of lysing the YAC target, indicating that they had some NK activity. Methylcholanthrene-induced sarcomas (MCA-102 and MCA-106) and the syngeneic EL-4 lymphoma were not lysed by these fresh splenocytes, indicating that they were resistant to lysis by NK cells. However, culture of these fresh splenocytes in IL-2 for 5 days generated lymphokine-activated killer (LAK) cells that were capable of killing the MCA-102 and MCA-106 sarcoma, the EL-4 lymphoma, YAK cells, and syngeneic lymphoblast cells, but not normal lymphocytes. None of these target cells were lysed by normal C57BL/6 splenocytes cultured in complete medium for 5 days. Much has been learned concerning AK cells in the mouse and the human. In the mouse, the precursor cells of the LAK phenomena are Thy 1^+ and nonadherent to plastic or nylon wool. The cell surface phenotype of the LAK effector cell is Thy 1^+ Lyt-1^-2^+. Cold target inhibition studies have revealed that the syngeneic tumor cells are lysed by the recognition of a deter-

TABLE 5.—Enhanced effect of adoptively transferred cells in the therapy of the FBL-3 lymphoma when systemic IL-2 is administered

Treatment group	Survival times (days)	Mean survival time ± SEM (days)	No. of cures/total No.
No treatment	15, 24, 24	21.0 ± 3.0	0/3
IL-2 alone*	17, 17, 19	17.6 ± 0.6	0/3
Expanded in vitro-sensitized cells	19, 19, 19, 24, > 80, > 60, > 60	37.3 ± 8.0	3/7
Expanded in vitro-sensitized cells + IL-2	> 60, > 60, > 60, > 60, > 60, > 60, > 60	60	7/7

* Purified IL-2 from Jurkat cell line.

TABLE 6.—Specificity of LAK-mediated lysis (*from* ref 36)

	% lysis ± SEM*		
	Effector cells		
Target cells	Fresh C57BL/6 splenocytes	C57BL/6 cultured in CM	C57BL/6 cultured in IL-2
MCA-102	1 ± 1	2 ± 2	53 ± 2
MCA-106	3 ± 1	4 ± 1	53 ± 1
EL-4	-20 ± 2	-18 ± 1	43 ± 2
C57BL/6 lymphocyte	-12 ± 3	-20 ± 5	8 ± 2
C57BL/6 Con A blast	3 ± 2	-10 ± 1	58 ± 5
YAC	20 ± 1	8 ± 1	72 ± 1

*Effector:target ratio = 100:1.

minant that is not present on normal lymphocytes or lymphoblast cells. The biologic role of these AK cells is not known, but we have hypothesized that they may play a role in immune surveillance against transformed cells (16). These cells are logical candidates for use in adoptive immunotherapy.

In preliminary experiments, it appears that these activated tumor cells are capable of inhibiting the growth of established pulmonary metastases from the B16 melanoma (Mazumder et al, unpublished data). In these experiments, B16 melanoma cells were injected iv and 3 days later, LAK cells were infused iv. Two weeks later, the mice were killed and the number of pulmonary metastases was counted in a blinded fashion. The results of a characteristic experiment are shown in table 7. Mice receiving infusion of LAK cells had far fewer metastases than mice receiving no treatment. The lungs of two mice, one treated with LAK cells and one untreated, are shown in figure 10.

HUMAN STUDIES

Very few clinical trials of adoptive immunotherapy have been performed in humans. Early trials involved empiric maneuvers such as infusion of spleen cells from normal allogeneic donors, infusion of thoracic duct lymphocytes from normal relatives immunized with tumor, infusion of large numbers of cultured B-lymphocyte lines, or infusion of lymph node cells from pigs immunized with human tumors (1). In light of recent developments in cellular immunology, these attempts had little hope of succeeding and complied with none of the criteria we now expect to be necessary for successful adoptive immunotherapy.

The treatment strategy outlined in the introduction and in table 1 has not been successfully tested in humans, although much work in this area is proceeding.

We have taken two approaches (suitable for adoptive transfer in humans) to generating lymphoid cells with specific antitumor reactivity. One approach is the generation of lymphoid cells with specific proliferative activity against human tumors using in vitro mixed lymphocyte tumor sensitization cultures. An alternative approach is the generation of AK cells with antitumor reactivity utilizing the peripheral blood lymphocytes of patients with cancer. These two approaches will be considered separately.

Generation of Autologous Lymphocytes With Antitumor Reactivity Using Mixed Lymphocyte Tumor Sensitization Cultures

Several groups have reported the generation of cells with selective antitumor reactivity in humans, using in vitro sensitization cultures of peripheral blood lymphocytes (PBL) co-incubated with autologous tumor cell preparations (32,37-39). Little is known, however, concerning the specificity of the cells that have been derived.

Several major challenges exist in the pursuit of these studies. The availability of suitable human tumor cell preparations remains a problem. Although tumor tissue can readily be obtained from surgically resected specimens, methods for preparing single cell suspensions from solid tumors are inadequate. The use of either mechanical

TABLE 7.—Treatment of established B16 melanoma pulmonary metastases with the systemic adoptive transfer of LAK cells

Treatment group*	No. of metastases per mouse	Mean No. of metastases
No treatment	3, 12, 12, 12, 15, 18, 22	13.4
Normal lymphocytes	45, 49, 64, 81, 101, 101, 142	83.3
LAK cells	34, 51, 59, 73, 108, 121, 161	86.7

*3 days after the injection of 2×10^5 B16 melanoma cells iv, 8×10^7 normal syngeneic splenocytes or LAK cells were injected iv.

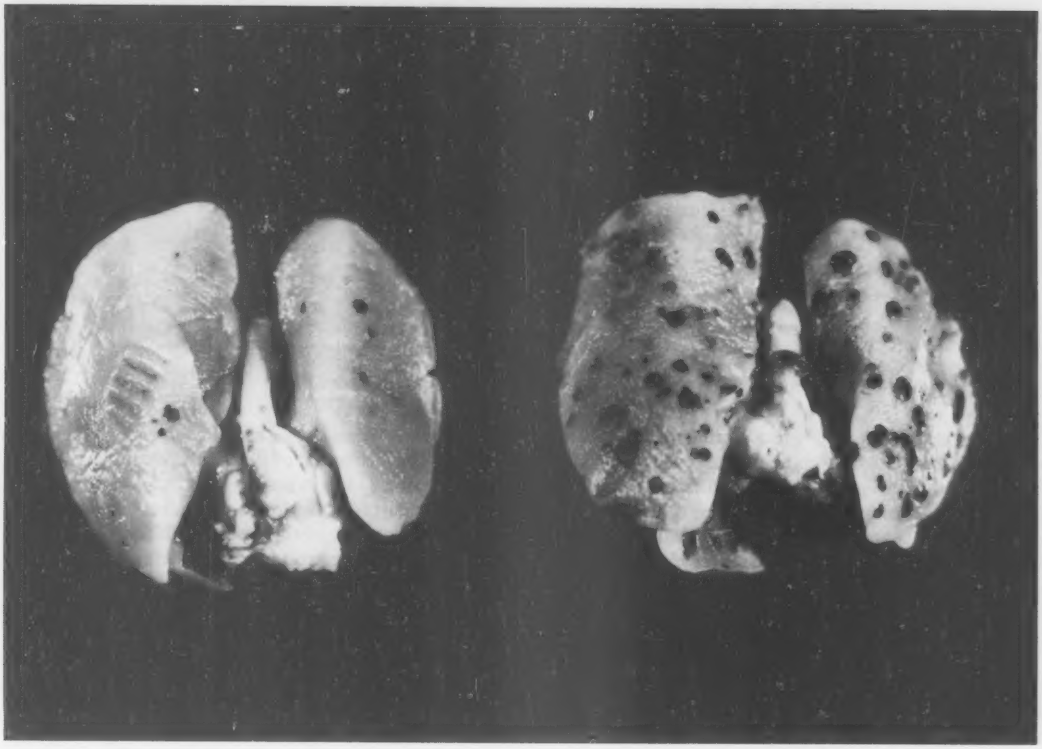


FIGURE 10.—C57BL/6 mice were injected iv with B16 melanoma cells and 3 days later were treated with syngeneic LAK cells. These cells significantly reduced the number of melanoma pulmonary metastases observed 15 days after tumor cell injection. The lungs on the left were obtained from a mouse treated with LAK cells. The lungs on the right were taken from a mouse receiving normal lymphocytes (from experiment shown in table 7).

or enzymatic dissociation techniques results in very low cell yields with poor viability. Virtually all tumor cell preparations are contaminated with macrophages, lymphocytes, and normal stromal cells and it is often difficult to distinguish between these elements cytologically. In addition, the availability of normal human tissue is quite limited. Autopsy material is usually not of suitable quality for these immunologic studies because of the rapid autolysis of tissue after death. In general, only small amounts of normal tissue are available from surgical specimens and the techniques for preparing single cell suspensions from normal tissue are also poor.

Extensive studies are underway to optimize techniques for the generation of reactive cells, using in vitro sensitization cultures of PBL co-incubated with autologous tumor. The single cell suspensions of tumor that we used in these studies were prepared by incubation of tumor fragments with hyaluronidase, collagenase, and deoxyribonuclease. Following this incubation, viable tumor cells were collected on Ficoll-Hypaque density gradients and were further purified by selective depletion of adherent mononuclear cells. A discontinuous three-step gradient to eliminate contaminating lymphocytes is also utilized when necessary. Utilizing this technique, satisfactory preparations of tumor cells can be obtained in approximately one-half of individuals. Varying proportions of responding lymphocytes and stimulator tumor cells are co-incubated for 5-7 days prior to assessing the proliferative response of these cultures, compared to suitable controls of lymphocytes and tumor cells incubated alone.

Of 37 autologous mixed lymphocyte tumor sensitization cultures performed, 19 exhibited statistically significant proliferation when compared to controls (Grimm et al, unpublished data). These proliferative responses were far lower than those generally obtained in standard allogeneic mixed lymphocyte cultures. Stimulation indices of positive mixed lymphocyte-tumor cultures generally were in the range of five to 15 and were rarely higher. An example of one such experiment, in which cultures of lymphocytes from a 40-year-old male with synovial cell sarcoma were co-incubated with his autologous tumor, is shown in table 8. In this experiment, 10^5 PBL were mixed with varying numbers of irradiated tumor cells. Stimulator cells ranged from 6×10^3 to 10^5

TABLE 8.—Mixed lymphocyte tumor interaction culture of a 40-yr-old male with synovial cell carcinoma

No. of cells × 10^{-5}				
Peripheral blood lymphocytes	Tumor cells	Mean cpm ± 1 SEM	Stimulation index	*P* value*
1	1	8582 ± 239	13.8	< 0.0025
1	0.5	3086 ± 329	6.9	< 0.005
1	0.25	926 ± 152	2.0	< 0.05
1	0.125	1232 ± 381	2.6	< 0.1
1	0.06	467 ± 57	0.9	> 0.1
1	0	434 ± 103	—	—
0	1	2608 ± 675	—	—
0	0.5	95 ± 15	—	—
0	0.25	77 ± 4	—	—
0	0.125	125 ± 18	—	—
0	0.06	83 ± 17	—	—

*Welch's 2-sample t-test for samples with unequal variances.

cells per microtiter well. In this experiment, lymphocytes alone incorporated 434 cpm. Tumor cells (10^5) alone incorporated 2608 cpm, although < 200 cpm were incorporated into all lower numbers of tumor cells. When mixing lymphocytes and tumor cells, stimulation indices of 13.8, 6.9, and 2.0 were seen at the three highest ratios of responders to stimulators. These stimulations, though modest, were statistically significant ($P < 0.0025$, $P < 0.005$, and $P < 0.05$, respectively).

Although the precise cell types required for adoptive immunotherapy are not known, our animal experiments suggested that cells with a proliferative response to tumor will be an important component of successful therapy (17,25). In our studies, we have therefore emphasized the identification of these proliferative cells. Immediately after performing mixed lymphocyte tumor sensitization cultures, these cells are cloned in an attempt to find individual clones with high levels of antitumor reactivity. Clones have been identified that proliferate when in contact with tumor, though the specificity of these reactions is unknown and is the subject of intensive study.

Activated Killer Cells

A variety of techniques for generating human AK cells have become available in recent years, including incubation with IL-2 (31-33), incubation with lectin (35,40), or activation by co-incubation with pooled allogeneic lymphocytes (34,41). In all cases, these activated lymphocytes can lyse fresh uncultured autologous and allogeneic tumor cells, but not normal peripheral blood lymphocytes. PBL from virtually all normal and tumor-bearing individuals appear to have the capacity to generate these cells. Little is known concerning the lymphocyte receptors involved in activated cell killing or the membrane components recognized by activated lymphocytes.

Perhaps the best-characterized activated killer system is the LAK system extensively studied by Grimm et al (32,33). An example of this phenomenon is shown in table 9. The incubation of PBL from patients with cancer with IL-2 for 2-3 days results in the generation of killer cells capable of lysing fresh autologous tumor cell preparations, but not fresh peripheral blood lymphocytes. The results of four experiments studying the kinetics of generation of LAK cells are shown in figure 11 (32).

A simple technique for generating AK cells is activation by the lectins Concanavalin (Con A) or Phytohemagglutinin (PHA) (35,40,42). In all three patients shown in figure 12, incubation of PBL with Con A resulted in the generation, after 2-3 days, of cells capable of killing autologous tumor, but with no lysis of autologous PBL or autologous lymphoblasts. The third technique that we have studied extensively for generating these activated killer cells is based on the original description of Zarling et al (43), who demonstrated that pool sensitization of PBL from leukemia patients resulted in the generating of cells able to lyse autologous leukemia cells. We have extended these observations to a variety of solid tumors. Examples of studies on two patients, one with breast cancer and one with melanoma, are shown in figure 13. As can be seen in the left-hand portion of this figure, lymphocytes which were co-incubated with a pool of allogeneic lymphocytes for 7 days resulted in the generation of cells capable of lysing autologous tumor, but not autologous PBL or lymphoblasts. Single allogeneic sensitization (fig 13, middle) was also capable of generating cells lytic for autologous tumor, although much weaker lytic cultures were generated than when pooled allogeneic sensitizations were performed.

The relationship among the different kinds of activated cells is presented in table 10. Although the serologic phenotype of the effector cell of PHA-activated killers (PAK), alloactivated killers (AAK), and LAK killers are identical, the serologic phenotype of the precursor cells is different. Both PAK and AAK cells are OKT3$^+$ and OKM1$^-$, whereas the LAK cell is OKT3$^-$ and OKM1$^-$. It appears likely that the final common pathway of all acti-

TABLE 9.—Lack of autologous fresh tumor lysis by fresh PBL (*from* ref 32)*

Patient No.	% specific lysis (± SEM)		
	K562 target fresh PBL effector	Autologous tumor target	
		Fresh PBL effector	LAK effector
30	64.2 ± 0.5	-3.7 ± 2.4	89.8 ± 1.6
	46.3 ± 0.5	-1.0 ± 3.6	63.4 ± 4.8
	19.0 ± 0.9	-1.6 ± 7.6	37.3 ± 4.6
37	44.0 ± 0.4	-0.9 ± 0.2	44.1 ± 2.3
	26.3 ± 1.3	-0.8 ± 0.2	34.1 ± 1.8
48	Not tested	Not tested	22.3 ± 1.4
	22.2 ± 0.2	2.0 ± 1.6	51.4 ± 2.8
	8.6 ± 0.2	0.0 ± 1.1	40.4 ± 2.0
	1.6 ± 0.2	3.4 ± 0.6	16.3 ± 0.8
52	14.0 ± 0.8	-3.6 ± 0.4	30.0 ± 0
	4.0 ± 0.7	-1.7 ± 0.6	21.0 ± 1.0
	1.0 ± 0.4	1.6 ± 1.1	13.0 ± 1.0
54	24.1 ± 0.0	2.4 ± 1.8	24.6 ± 1.9
	14.9 ± 0.8	3.7 ± 2.5	9.3 ± 0.4
	7.3 ± 0.8	1.8 ± 0.2	4.8 ± 0.1
56	24.6 ± 2.2	-3.2 ± 3.2	38.6 ± 8.9
	7.5 ± 0.0	3.4 ± 4.0	19.2 ± 7.2
	1.7 ± 0.3	3.9 ± 0.9	6.1 ± 8.3
57	27.8 ± 1.2	-7.9 ± 0.4	50.2 ± 0.7
	11.4 ± 1.1	-3.4 ± 1.0	14.6 ± 1.2
	3.3 ± 0.4	1.8 ± 3.7	5.1 ± 1.1
58	59.9 ± 3.3	-5.4 ± 0.6	27.5 ± 4.7
	27.3 ± 0.5	0.4 ± 5.6	18.4 ± 0.7
	8.8 ± 0.4	-4.4 ± 2.7	3.5 ± 1.6
63	39.3 ± 0.3	-0.6 ± 0.8	43.0 ± 1.4
	14.4 ± 0.2	5.6 ± 4.3	26.2 ± 3.4
	3.8 ± 0.2	1.9 ± 0.8	7.6 ± 0.4
67	34.9 ± 0.9	1.0 ± 1.0	16.1 ± 1.1
	14.5 ± 0.7	-3.6 ± 1.1	11.5 ± 1.9
	4.7 ± 0.1	2.8 ± 0.9	7.2 ± 0.9

*Fresh PBL were used at effector to target ratios of 100:1, 25:1, and 6:1; PBL were cultured in IL-2 to produce LAK, which were tested for lysis at effector to target ratios of 40:1, 10:1, and 2.5:1.

vated cell killing is the exposure of lymphocytes to IL-2. The serologic phenotypes assigned in table 10 were determined on the basis of depletion studies with monoclonal antibody and complement. The dependence on $OKT3^+$ cells in the generation of PAK and AAK killing, but not LAK killing, may in fact be due to the requirement of $OKT3^+$ cells for the production of IL-2 during the activation cultures.

A clearer understanding of the specificity of activated cell killing is necessary. In preliminary experiments, normal cell suspensions from liver, lung, and other normal organs are not lysed by AK cells.

Extensive studies are underway to attempt to understand the nature of activated cell killing and its biologic significance. Activated cell killing appears to be a lytic system quite distinct from that of classic cytotoxic T-lymphocyte killer cells (CTL) or NK cells. A comparison of these lytic systems is presented in table 11. The precursor of the LAK cell is distinct from the precursor of NK and CTL cells. Whereas NK cells bear the OKM1, OKT11, and Leu 7 markers and CTL precursors bear the OKT3 and Leu 1 markers, none of these phenotypic markers are present on the precursors of LAK cells. The serologic phenotype of the effector cell involved in LAK killing is, however, quite similar to that of the phenotype of CTL and quite different from that of NK cells. For this reason, it is likely that the LAK cell represents a cell in the differentiation lineage of CTL.

The biologic role of activated killer cells is unknown. These cells may play a role in immunosurveillance by eliminating transformed cells or cells expressing non-self determinants in vivo. Since human PBL are constantly being activated by a variety of environmental antigens, the ability of an activated lymphocyte to recognize fresh tumor cells might well confer a selective survival advantage.

It is important to emphasize that these AK cells kill fresh autologous tumor cell preparations. In this respect, these AK cells are very different from classical NK cells. NK cells can lyse only in vitro cultured cell lines and cannot lyse fresh autologous tumor cells. It is for this reason that AK cells are more likely candidates than NK

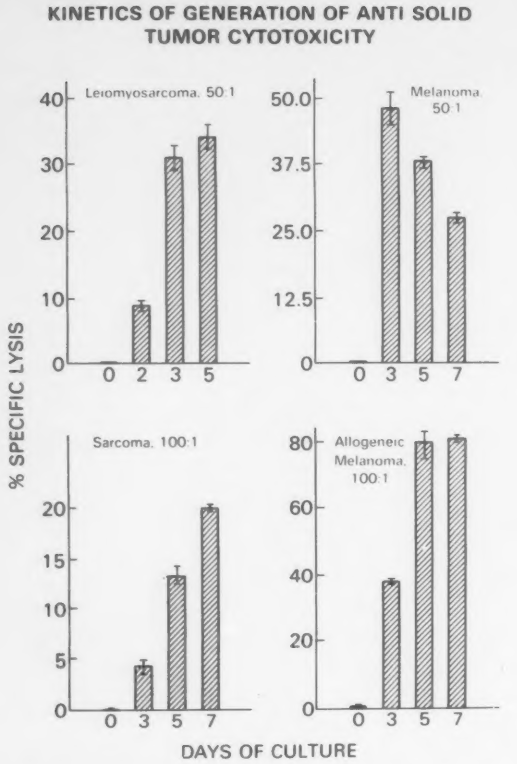


FIGURE 11.—The kinetics of LAK cell development from 3 patients. The bottom panels show the kinetics of activation of LAK cells from a single patient directed against the autologous tumor (left) and the allogeneic tumor (right). Note that in all cases, LAK cells are generated within 3 days of incubation in IL-2-containing supernatants (ref 32).

cells for a biologic role in antitumor immune host reactions. AK cells may play a role in adoptive immunotherapy and may form an important class of effector cells for the adoptive immunotherapy of cancer in humans.

Preliminary Studies of the Infusion of Activated Killer Cells in Man

As in vitro work techniques for generating cells required for adoptive immunotherapy have progressed, we have also begun studies of the feasibility and toxicity of the infusion of large numbers of activated lymphoid cells in humans (22,44).

Our initial studies of AK cells were performed in patients with large tumor burdens who had failed all standard therapy. We initially treated three patients with autologous LAK cells (22). In our first study, between 1.5 to 5 × 10^8 PBL activated with IL-2-containing supernatants were infused into autologous patients. There was no recognizable toxicity associated with the infusion of this small number of cells and no positive therapeutic effects were seen. Chemical studies of hepatic, renal, and hematologic parameters were normal and pulmonary function studies revealed no changes after the cell infusions. In these studies, cells were labeled with either indium 111 or gallium 67 prior to injection. Three separate techniques were used to monitor the in vivo traffic of these injected cells. External point counts were obtained over vital organs using a collimated whole-body scanner. Rectilinear scanning using a whole-body imager and a computer-interfaced gamma camera were also used to measure LAK cell distribution. The results of these cell distribution studies are shown in figures 14 and 15. The absence of toxicity seen in these initial trials encouraged us to perform a second generation of clinical trials, again in advanced cancer patients, designed to test the feasibility and toxicity of the infusion of larger numbers of AK cells (44).

For these later clinical trials, we used PAK cells as the most practical method for producing large numbers of AK cells. At the time these trials were conceived, IL-2 was not available in amounts sufficient to generate the very large numbers of LAK cells required for these studies. In these trials, patients with large tumor burdens who had failed standard therapies were leukapheresed, and the lymphocytes were activated with PHA for 2-3 days and were then infused iv. Immediately prior to each infusion, another leukapheresis was performed to obtain cells for the next infusion 2-3 days later. In this sequential fashion, patients were leukapheresed and reinfused with activated cells three times per week, up to 15 times. A summary of ten patients included in this trial is shown in table 12. As can be seen, some patients received up to 17 × 10^{10} activated lymphoid cells.

The feasibility of utilizing sequential leukapheresis and reinfusion of large numbers of activated lymphocytes was demonstrated in this clinical study. Toxicity encountered with these cell infusions included fever and chills in all patients, headaches in five of ten, and nausea and vomiting in three of ten. Rbc transfusions were required in eight of ten patients due to rbc loss in the multiple leukaphereses. No evidence for any autoimmune disease or abnormal serum chemical or coagulation studies was found. A transient decrease in the diffusion capacity of the lung, that returned to normal 24 hours later, was seen immediately following infusion.

The in vivo traffic of PAK cells was studied in two patients, using indium 111-labeled cells (44). About 70% of the initial counts remained in the body for 2 weeks. A distribution pattern quite similar to that seen for LAK cells was observed following the first infusion. In the initial infusion, the cells migrated preferentially to the spleen and liver after a short localization in the lungs. However, after the ninth infusion, cell traffic studies revealed a dif-

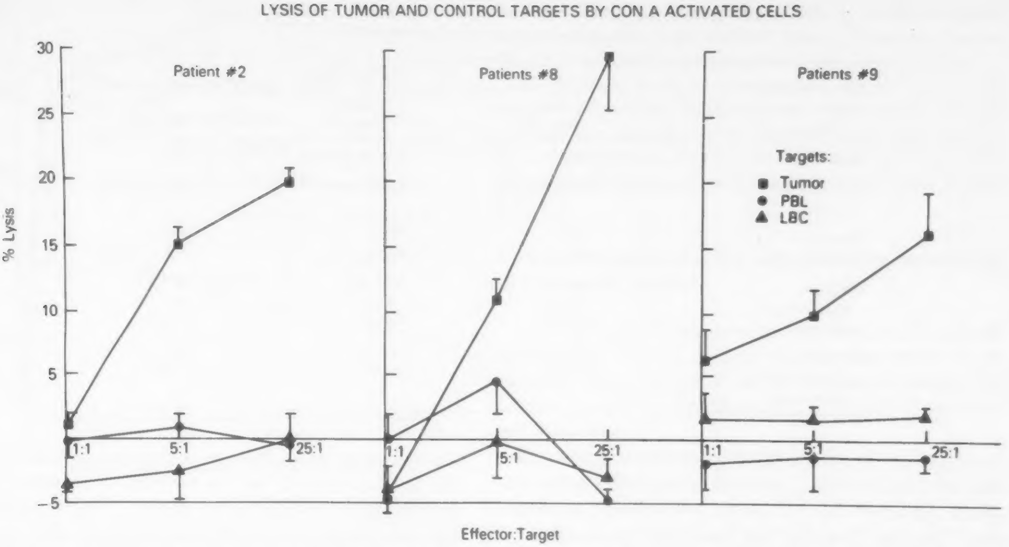


FIGURE 12.—The generation of Con A-activated killer cells in 3 different cancer patients. Peripheral blood lymphocytes were incubated with Con A and tested for lysis of autologous tumor, autologous PBL, or autologous lymphoblasts. Note that in all cases, only autologous tumor was lysed by these Con A-activated killer cells (ref 35).

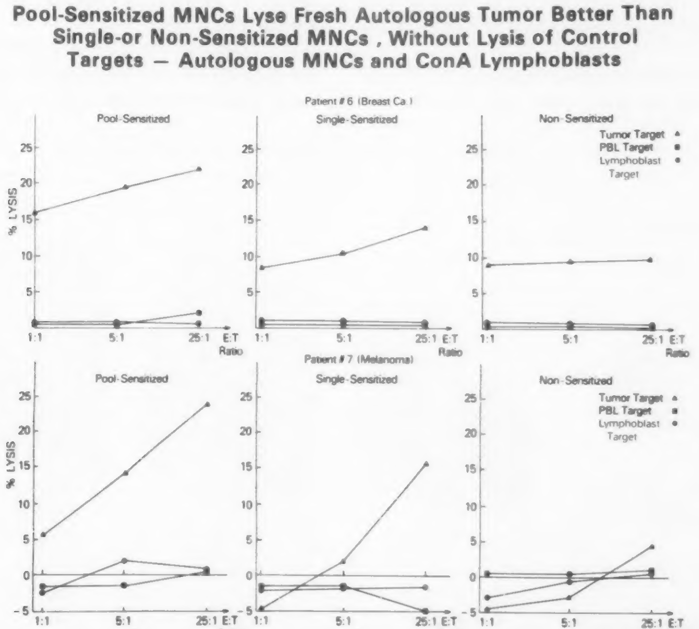


FIGURE 13.—The generation of AAK cells from 2 cancer patients. The left panel shows the results of a pool-sensitization, the middle panel shows the results of a single sensitization, and the right panel shows cells incubated only in medium. Note that pool-sensitized cells develop the ability to kill autologous tumor target cells, but not autologous PBL or lymphoblast targets. Single sensitized cells also generate lysis for autologous tumor, but at a lower level than that seen in pool-sensitized cells (ref 34).

TABLE 10.—Characteristics of the 3 activation systems generating killing activity for NK-resistant solid tumor cells (*from* ref 16)

Characteristic (development kinetics)	Lytic activity		
	PAK (Day 1,2)	AAK (Day 2,3)	LAK (Day 2,3)
Stimulus	PHA	Allogeneic cells	IL-2
Specificity of cytotoxicity	Fresh tumors	Fresh tumors	Fresh tumors
Precursor location	TDL+ PBL+	TDL+ PBL+	TDL+ PBL+
Serologic phenotype of effectors	OKT.8+ OKT.3+ OKM.1-	OKT.8+ OKT.3+ OKM1-	OKT.8+ OKT.3+ OKM1-
Serologic phenotype of precursors	OKT.3+ OKM.1-	OKT.3+ OKM.1-	OKT.3- OKM.1-

ferent pattern of distribution (fig 16). At 24 and 96 hours after the initial infusion, the ratio of counts in the liver to that in the lungs was 13.8 and 12, respectively. After the ninth infusion, however, an increased distribution of counts to the lungs was seen and the liver to lung ratio of counts at 24 and 96 hours was 1.3 and 1.6, respectively. This increased traffic of cells to the lungs in the final infusion probably represents a saturation of the ability of the spleen and liver to clear these activated cells from the circulation.

To test for the presence of activated cells in the circulation, several approaches were taken (44). Before each leukapheresis, fresh PBL were obtained by phlebotomy and incubated for 2 days in medium alone, prior to assessing uptake of tritiated thymidine. In all nine patients who were tested, a dramatic increase in the spontaneous proliferation of unstimulated cells was seen. This increase was further enhanced by the addition of lectin-free IL-2 to the 2-day assay. Far more of these cells were present in the circulation after the late infusions than after the early infusions.

We further assessed the presence of circulating activated cells by measuring the ability of fresh PBL to lyse fresh autologous tumor cells in vitro. Figure 17 shows

TABLE 11.—Contrasting attributes of LAK cells, NK cells, and CTL (*from* ref 16)

Characteristic	Lytic activity		
	NK	LAK	CTL
Development kinetics	Fresh PBL	Expressed by Days 2,3	Not expressed until Days 5,6
Stimulus	None, interferon augments	IL-2	Specific antigen
Specificity of cytotoxicity	Bone marrow; leukemia cells	Fresh solid tumors (+ all NK targets)	Specific antigen-expressing cells
Precursor location	TDL- PBL+	TDL+, spleen+ PBL+, tumor+ BM+	TDL unknown PBL+
Serologic phenotype of effector	OKM1+ OKT3- OKT8-	OKM1- OKT3+ OKT8+ LEU1+ OKT4- 4F2+	OKM1- OKT3+ OKT8+ LEU1+ OKT4- 4F2+
Serologic phenotype of precursor	OKM1+ OKT3- LEU1- OKT11+ LEU7+	OKM1- OKT3- LEU1- OKT9- OKT10- TAC- OKT11-	OKM1- OKT3+ LEU1+

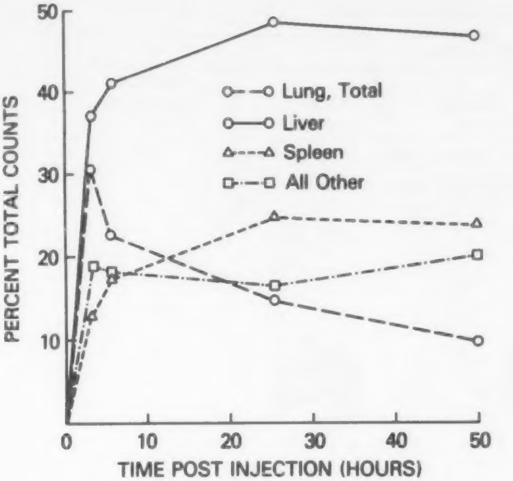


FIGURE 14.—Serial gamma camera views were obtained to determine the organ distribution of indium 111-labeled LAK cells. The total activity in lung, spleen, liver, and other body areas is expressed as a % of the total counts in the gamma camera fields. Note that after initial distribution to the lungs, these cells accumulate in the liver and spleen (ref 22).

that the lysis of fresh tumor cells by PBL increased with successive leukaphereses. We attribute this increase to an increase in AK cells circulating after the repeated infusions. These are the only patients in whom we have consistently seen high levels of killer cells in the circulation that are capable of lysing fresh autologous tumor.

All patients in these studies had tumor burdens of several kilograms. Although no therapeutic clinical effects were seen in any of these patients, these studies have demonstrated that it is feasible to infuse large numbers of autologous activated lymphoid cells with minimal toxicity.

Future Prospects for the Adoptive Immunotherapy of Cancer in Humans

Although considerable progress is being made in both murine and human studies, a great deal needs to be learned before successful adoptive immunotherapy for human cancer is a clinical reality. A variety of unsolved problems exist.

Perhaps the major challenge is the development of methods for generating autologous cell populations with selective reactivity to tumor cells. The most productive approach for obtaining these cells is not yet clear, although many techniques are being investigated. In vitro mixed lymphocyte tumor interactions might yield cells with specific antitumor reactivity, although studies to modify and optimize the mixed lymphocyte tumor interaction techniques are necessary. A variety of modifications of the responder and stimulator cell populations may improve the reactivity and specificity of the re-

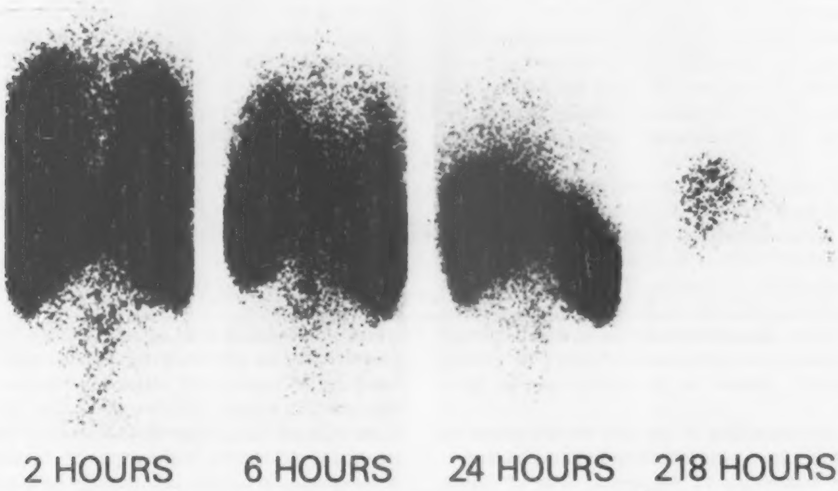


FIGURE 15.—Sequential rectilinear scans of a patient following injection of indium 111-labeled LAK cells. At 2 hrs, counts are seen in the lungs, liver, and spleen. At 6 hrs, label clears from the lungs and concentrates in the liver and spleen (ref 22).

TABLE 12.—Composite of patient characteristics (*from* ref 44)

	Characteristic				
Patient No.	Age	Diagnosis	Evaluable disease	No. of infusions	Total No. of cells infused ($\times 10^{-10}$)
1	22	Synovial cell sarcoma	Lung	9	7.2
2	37	Epithelioid sarcoma	Lung	9	5.5
3	47	Melanoma	Skin	9	2.8
4	36	Osteosarcoma	Lung	9	1.9
5	39	Colorectal carcinoma	Liver	14	17.1
6	51	Colorectal carcinoma	Liver	15	7.3
7	60	Colorectal carcinoma	Lung	15	8.4
8	42	Fibrosarcoma	Lung	14	10.1
9	29	Undifferentiated sarcoma	Lung	9	3.2
10	37	Synovial cell sarcoma	Lung	7	1.6

sulting sensitized cells. Depletion of adherent cells, selective depletion of suppressor cells, the addition of growth factors to enhance the sensitization process, and altering the immunogenicity of the stimulator tumor cells by chemical modification or mutagenesis are all approaches that require study. The cloning of specifically reactive cells is also a valuable tool that can be exploited in these efforts. If animal studies can accurately identify the cell types necessary in humans, then selected techniques for cloning the appropriate cells can be developed to insure that the infused populations will have the highest possible reactivity. The development of reliable in vitro correlates of in vivo effectiveness would be extremely valuable.

Additional work is required to improve the techniques for the long-term growth of human cells. Whereas murine cell cultures can usually be grown indefinitely, cultures of human T cells in IL-2 generally stop growing after approximately 3 months. The reason for this "crisis" in growth is not known. Because rodent cells will grow indefinitely, this problem should be resolvable and probably represents a nutritional deficiency or growth factor deficiency present in the media used to grow human cells.

A clearer understanding of the host modifications required to enable successful adoptive immunotherapy is needed. The elimination of suppressor cells by either cyclophosphamide or irradiation appears necessary to enable adoptively transferred cells to mediate the regression of established murine tumors in several models. Few good assays for suppressor cells exist in the human that can be used to determine the optimal techniques for host modification prior to adoptive immunotherapy. Hopefully, either cyclophosphamide or irradiation will be suitable for use in the human for this purpose, although this is speculative.

Preliminary information suggests that the ability of expanded cells to function in adoptive immunotherapy in murine models can be enhanced by the simultaneous infusion of IL-2. The recent availability of IL-2 from cloned genes expressed in bacteria will provide the large amounts of IL-2 required to test whether immunotherapeutic effects can be enhanced by infusion of this lymphokine in humans.

One of the major advantages of adoptive immunotherapy is the ease with which it can be used in conjunction with other therapies. Careful studies in murine systems will be necessary to determine which therapies synergize with the adoptive transfer of cells in mediating anticancer effects.

A major characteristic of the immune system is the specificity of its reactivity. If immune cells can be isolated in the human with selective antitumor reactivity, then existing animal models suggest that the infusion of these cells can have important therapeutic benefit in the treatment of cancer without major toxicity. Modern developments in cellular immunology have provided a rational means for developing such adoptive immunotherapies in humans. Although immunotherapy has thus far added little to the effective treatment of human

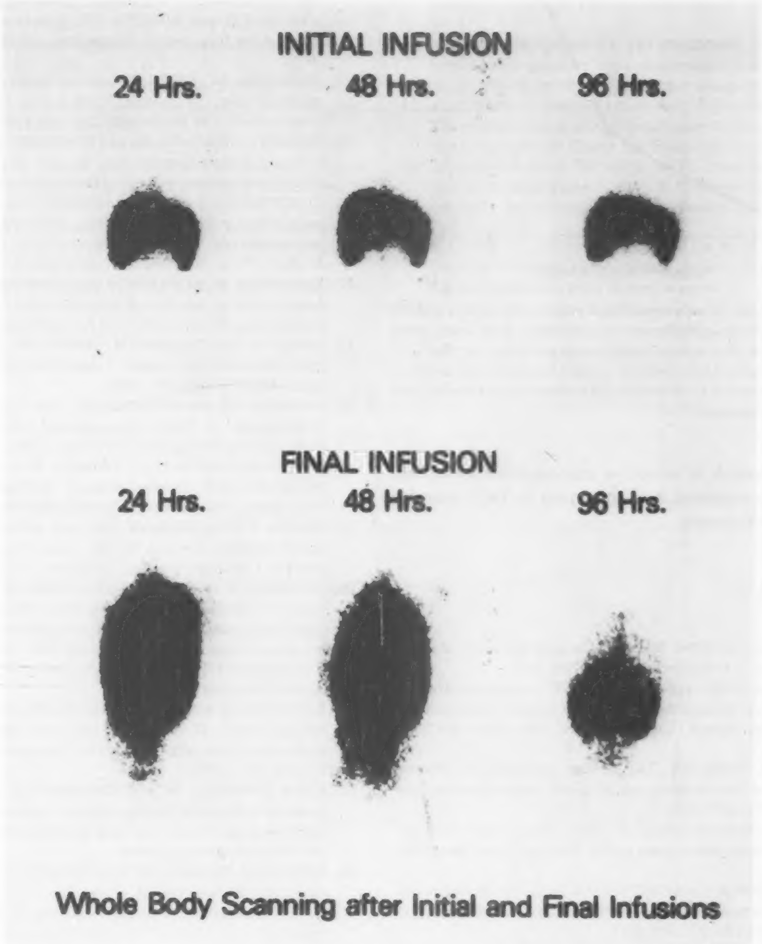

INITIAL INFUSION
24 Hrs.
48 Hrs.
96 Hrs.
FINAL INFUSION
24 Hrs.
48 Hrs.
96 Hrs.
Whole Body Scanning after Initial and Final Infusions

FIGURE 16.—Full-body rectilinear scanning at various times after the initial (top) and the 9th (bottom) infusion of indium 111-labeled PAK cells. An increased distribution of cells to the lungs compared to the liver and spleen was seen in the final infusion, when compared to the 1st infusion. The patient had a tumor mass in the left thigh, which was also visualized after the final infusion (ref 44).

7

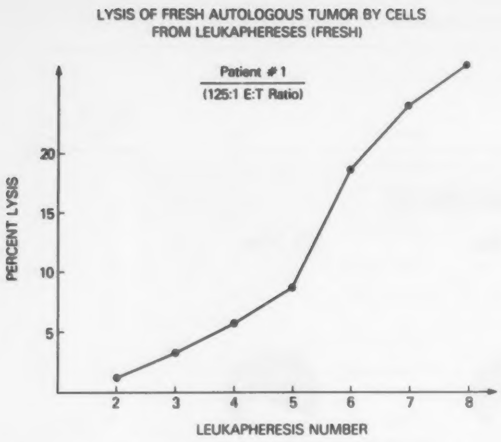


FIGURE 17.—The lysis of autologous fresh tumor cells from a patient who received multiple leukaphereses of autologous PAK cells. Fresh PBL were obtained prior to each leukapheresis and tested for their ability to lyse autologous tumor cells in a 4-hr chromium release assay. Note that an increased level of lysis of autologous tumor was seen after successive leukaphereses (ref 44).

cancer, the approach of adoptive immunotherapy is only beginning to be explored and continues to hold promise for further effectiveness.

REFERENCES

1. ROSENBERG SA, and TERRY W. Passive immunotherapy of cancer in animals and man. Adv Cancer Res 25:323-388, 1977.
2. FEFER A, CHEEVER MA, and GREENBERG PD. Lymphocyte transfer as potential cancer immunotherapy. *In* Immunological Approaches to Cancer Therapies (Mihich E, ed). New York, John Wiley and Sons, 1982, p 333.
3. KEDAR E, and WEISS DW. The in vitro generation of effector lymphocytes and their employment in tumor immunotherapy. Adv Cancer Res 38:171-287, 1983.
4. ALEXANDER P. Immunotherapy of cancer. Experiments with primary tumors and syngeneic tumor grafts. Prog Exp Tumor Res 10:22, 1968.
5. FEFER A, EINSTEIN AB, and CHEEVER MA. Adoptive chemoimmunotherapy of cancer in animals: a review of results, principles and problems. Ann NY Acad Sci 277:492, 1976.
6. BORBERG H, OETTGEN HF, CHOUDRY K, ET AL. Inhibition of established transplants of chemically induced sarcomas in syngeneic mice by lymphocytes from immunized donors. Int J Cancer 10:539, 1972.
7. SMITH HG, HARMEL RP, HANNA MG, ET AL. Regression of established intradermal tumors and lymph node metastases in guinea pigs after systemic transfer of immune lymphoid cells. JNCI 58:1315, 1977.
8. BERENDT MJ, and NORTH RJ. T-cell mediated suppression of antitumor immunity. An explanation for progressive growth of an immunogenic tumor. J Exp Med 151:59, 1980.
9. FERNANDEZ-CRUZ E, HALLIBURTON B, and FELDMAN JD. In vivo elimination by specific effector cells of an established syngeneic rat Moloney virus-induced sarcoma. J Immunol 123:1772, 1979.
10. CHEEVER MA, KEMPF RA, and FEFER A. Tumor neutralization, immunotherapy, and chemoimmunotherapy of a Friend leukemia with cells secondarily sensitized in vitro. J Immunol 119:714, 1977.
11. EBERLEIN TJ, ROSENSTEIN M, SPIESS P, ET AL. Adoptive chemoimmunotherapy of a syngeneic murine lymphoma using long term lymphoid cell lines expanded in T cell growth factor. Cancer Immunol Immunother 13:5-13, 1982.
12. EBERLEIN TJ, ROSENSTEIN M, and ROSENBERG SA. Regression of a disseminated syngeneic solid tumor by systemic transfer of lymphoid cells expanded in IL-2. J Exp Med 156:385-397, 1982.
13. MORGAN DA, and RUSCETTI FW. Selective in vitro growth of T lymphocytes from normal human bone marrows. Science 193:1007, 1976.
14. ROSENBERG SA, SPIESS PJ, and SCHWARZ S. In vitro growth of murine T cells. IV. Use of T cell growth factor (TCGF) to clone lymphoid cells. Cell Immunol 54:293-306, 1980.
15. LOTZE MT, STRAUSSER JL, and ROSENBERG SA. In vitro growth of cytotoxic human lymphocytes. II. Use of T cell growth factor (TCGF) to clone human T cells. J Immunol 124:2972-2978, 1980.
16. ROSENBERG SA, GRIMM EA, LOTZE MT, ET AL. The growth of human lymphocytes in T-cell growth factor: potential applications to tumor immunotherapy. *In* Lymphokines (Mizel SB, ed). New York, Academic Press, Inc, 1982, vol 7, pp 213-247.
17. ROSENSTEIN M, EBERLEIN TJ, and ROSENBERG SA. Adoptive immunotherapy of established syngeneic solid tumors: role of T lymphoid subpopulations. Submitted for publication.
18. LOVELAND BE, HOGARTH PM, CEREDIG RH, ET AL. Cells mediating graft rejection in the mouse. I. Lyt-1 cells mediate skin graft rejection. J Exp Med 153:1044, 1981.
19. LOVELAND BE, and MCKENZIE IFC. Cells mediating graft rejection in the mouse. II. The Ly phenotypes of cells producing tumor allograft rejection. Transplantation 33:174, 1982.
20. ENGERS HD, SORENSON GD, TERRES G, ET AL. Functional activity in vivo of effector T cell populations. I. Antitumor activity exhibited by allogeneic mixed leukocyte culture cells. J Immunol 129:1292.
21. ENGERS HD, GLASEBROOK AL, and SORENSON GD. Allogeneic tumor rejection induced by the intravenous injection of Lyt-2^+ cytolytic T lymphocyte clones. J Exp Med 156:1280, 1982.
22. SHAMBERGER RC, ROSENBERG SA, SEIPP CA, ET AL. The effects of high dose methotrexate and vincristine on ovarian and testicular functions in patients undergoing postoperative adjuvant treatment for osteosarcoma. Cancer Treat Rep 65:739-746, 1981.
23. SUGARBAKER PH, and CHANG AE. Uncovered skin grafts in mice. J Immunol Methods 31:167, 1979.
24. ROSENSTEIN M, EBERLEIN T, KEMENY MM, ET AL. In vitro growth of murine T cells. VI. Accelerated skin graft rejection caused by adoptively transferred cells expanded in T cell growth factor. J Immunol 127:566-571, 1981.
25. KIM B, ROSENSTEIN M, and ROSENBERG SA. Clonal analysis of the lymphoid cells mediating skin allograft rejection; cloned Ly 1^+2^- proliferative, noncytotoxic long term cell lines mediate graft rejection in vivo. Transplantation, in press.
26. ROSENSTEIN M, EBERLEIN T, SCHWARZ S, ET AL. Simplified techniques for the isolation of alloreactive cell lines and clones with specific cytotoxic or proliferative activity. J Immunol Methods, in press.
27. EBERLEIN TJ, ROSENSTEIN M, SPIESS PJ, ET AL. The generation of long term T-lymphoid cell lines with specific cytotoxic reactivity for a syngeneic murine lymphoma. JNCI 69:109-116, 1982.
28. ROSENSTEIN M, and ROSENBERG SA. T lymphoid cell clones with lytic and proliferative activity to syngeneic tumor. Submitted for publication.
29. DONOHUE JH, and ROSENBERG SA. The fate of interleukin-2 following in vivo administration. J Immunol 130:2203-2208, 1983.
30. YRON I, WOOD TA, SPIESS P, ET AL. In vitro growth of murine T cells. V. The isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. J Immunol 125:238-245, 1980.
31. LOTZE MT, GRIMM E, MAZUMDER A, ET AL. In vitro growth of cytotoxic human lymphocytes. IV. Lysis of fresh and cultured autologous tumor by lymphocytes cultured in T cell growth factor (TCGF). Cancer Res 41:4420-4425, 1981.

32. GRIMM EA, MAZUMDER A, ZHANG HZ, ET AL. The lymphokine activated killer cell phenomenon: lysis of NK resistant fresh solid tumor cells by IL-2 activated autologous human peripheral blood lymphocytes. J Exp Med 155:1823-1841, 1982.
33. GRIMM EA, RAMSEY KM, MAZUMDER A, ET AL. Lymphokine-activated killer cell phenomenon. II. The precursor phenotype is serologically distinct from peripheral T lymphocytes, memory CTL, and NK cells. J Exp Med 157:884-897, 1983.
34. STRAUSSER JL, MAZUMDER A, GRIMM EA, ET AL. Lysis of human solid tumors by autologous cells sensitized in vitro to alloantigens. J Immunol 127:266-271, 1981.
35. MAZUMDER AM, GRIMM EA, ZHANG HZ, ET AL. Lysis of fresh human solid tumors by autologous lymphocytes activated in vitro with lectins. Cancer Res 42:913-918, 1982.
36. ROSENSTEIN M, YRON I, KAUFMANN Y, ET AL. Lymphokine activated killer cells: lysis of fresh syngeneic NK-resistant murine tumor cells by lymphocytes cultured in Interleukin-2. Cancer Res, in press.
37. KLEIN E, and VANKY F. Natural and activated cytolytic lymphocytes which act on autologous and allogeneic tumor cells. Cancer Immunol Immunother 11:183-188, 1981.
38. VANKY F, GORSKY T, GORSKY Y, ET AL. Lysis of tumor biopsy cells by autologous T lymphocytes activated in mixed cultures and propagated with T cell growth factor. J Exp Med 155:83-95, 1982.
39. VOSE B, and BONNARD GD. Human tumor antigens defined by cytotoxicity and proliferative responses of cultured lymphoid cells. Nature 296:359-361.
40. MAZUMDER A, GRIMM EA, and ROSENBERG SA. Characterization of the lysis of fresh human solid tumors by autologous lymphocytes activated in vitro with phytohemagglutinin. J Immunol 130:958-964, 1983.
41. MAZUMDER A, GRIMM EA, and ROSENBERG SA. The lysis of fresh human solid tumors by autologous lymphocytes activated in vitro by allosensitization. Cancer Immunol and Immunother 15:1-10, 1983.
42. MAZUMDER A, ROSENSTEIN M, and ROSENBERG SA. Lysis of fresh NK-resistant tumor cells by lectin activated syngeneic and allogeneic murine splenocytes. Cancer Res. Submitted for publication.
43. ZARLING JM, BACH FH, and KUNG PC. Sensitization of lymphocytes against pooled allogeneic cells. II. Characterization of effector cells cytotoxic for autologous lymphoblastoid cell lines. J Immunol 126:375, 1981.
44. MAZUMDER A, EBERLEIN TJ, GRIMM EA, ET AL. Phase I study of the adoptive immunotherapy of human cancer with lectin activated autologous mononuclear cells. Cancer, in press.

Monoclonal Antitumor Antibodies in the Lymphatics

John N. Weinstein,[1,*] Michael A. Steller,[1] David G. Covell,[1] Oscar D. Holton, III,[1] Andrew M. Keenan,[2] Susan M. Sieber,[3] and Robert J. Parker[3]

Monoclonal antitumor antibodies are being administered iv in a number of medical centers for diagnosis or therapy of human cancers. However, when the objective is to detect or to treat tumor in regional lymph nodes, sc injection may prove more effective. After sc injection, monoclonal antibodies enter local lymphatic capillaries, pass to the draining lymph nodes, and bind to target cells there. Antibody not bound in the first node group encountered passes to more distant nodes. If still not removed from the lymph flow, antibody passes into the bloodstream, principally through the thoracic duct. Initial studies of the lymphatic approach were done with antibodies directed against antigens on normal cell types in the mouse lymph node. The use of antibodies to normal cells made it possible to study the pharmacokinetics of delivery in a reproducible, quantitatively interpretable system. The pharmacologic models developed were then applied to the design of experiments on line 10 hepatocarcinoma of guinea pigs. As little as 2 mg of line 10 tumor could be identified by gamma camera imaging in regional nodes after injection of ^{125}I-labeled antibody. The uptake was immunologically specific, and autoradiography showed localization exclusively within the metastatic tumor. When the aim is to diagnose or treat early tumor metastases in the nodes, the lymphatic route can be expected to provide higher sensitivity, lower background, lower systemic toxicity, and faster localization than the iv route. Perhaps most interesting, the lymphatic route minimizes exposure of antibody to cross-reactive antigen present on normal tissues elsewhere in the body. Antitumor antibodies rejected for iv use because they also bind to normal tissues may, therefore, be useful in the diagnosis and treatment of lymph node metastases. **[Cancer Treat Rep 68:257–264, 1984]**

If this silver anniversary issue is viewed as an amalgam of progress reports on the last 25 years and harbingers of the next 25, the present paper definitely falls into the latter category. Clinical studies of monoclonal antibodies in the lymphatics are just beginning. However, the general aims of immunodiagnosis and immunotherapy are not new. They are rooted in the 19th century studies of Von Behring (1) on diphtheria antitoxin and of Hericourt and Richet (2) on antisera against osteogenic sarcoma. Paul Ehrlich, the patron saint of the field, argued at the turn of the century for the use of "bodies which possess a particular affinity for a certain organ . . . as a carrier by which to bring therapeutically active groups to the organ in question" (3). Further developments in the diagnostic and therapeutic use of antisera between 1920 and 1975 are associated with such investigators as Witebsky (4), Hirzfeld et al (5), Pressman and Korngold (6), Bale et al (7), DeCarvalho and Rand (8), Ghose et al (9), Order et al (10), and Goldenberg et al (11).

In 1975, Kohler and Milstein (12) introduced their technique for production of monoclonal antibodies. That development, in addition to broad-based advances in cell biology, immunology, and molecular biology, has given impetus to a new generation of studies on the delivery of antibodies to tumor targets. It is not that polyclonal antisera are clearly inferior to monoclonal antibodies for diagnostic and therapeutic use; many antisera have the advantage of reactivity with more determinants per cell. However, it is difficult to interpret experiments done with such heterogeneous, poorly characterized reagents; monoclonals have provided the uniformity required for incisive investigation.

---

[1] Laboratory of Mathematical Biology, Division of Cancer Biology and Diagnosis, National Cancer Institute, Bethesda, MD.

[2] Department of Nuclear Medicine, Clinical Center, National Institutes of Health, Bethesda, MD.

[3] Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, MD.

[*] *Reprint requests to:* John N. Weinstein, MD, Laboratory of Mathematical Biology, Division of Cancer Biology and Diagnosis, National Cancer Institute, Bldg 10, Rm 4B54, National Institutes of Health, Bethesda, MD 20205.

For diagnostic purposes, monoclonal antibodies can be labeled with gamma-emitting radionuclides for scintigraphic imaging. For therapy, a drug, toxin, or radionuclide can be coupled to the antibody; alternatively, a cytotoxic monoclonal itself may be used to mark tumor cells for destruction by host defenses. To date, the focus of work in vivo has been on iv administration. That emphasis is sensible because the bloodstream is a means of access to almost all organs in the body except the central nervous system. However, other possible routes of administration should not be ignored.

The lymphatic system is of undoubted importance in the spread of many malignancies, most prominently the carcinomas. Aside from its immunologic functions, the system is responsible for uptake and return to the circulation of serum proteins which have leaked out of the blood vessels. That physiologic function is reflected in the behavior of the lymphatics as a route of administration for pharmacologic agents, among them antibodies.

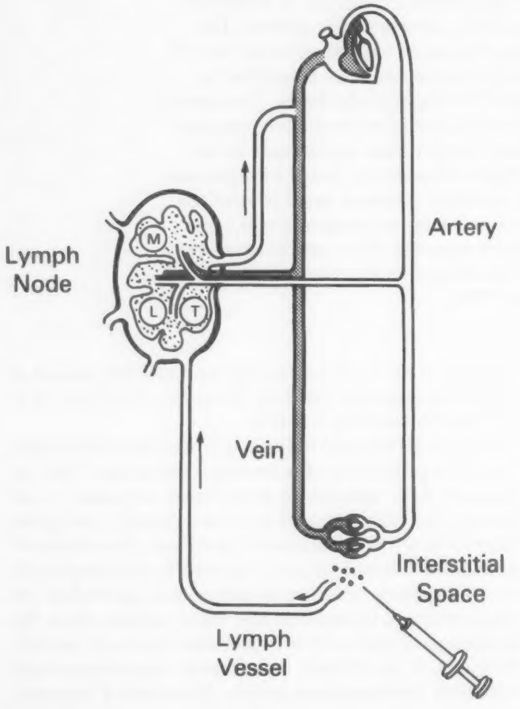


FIGURE 1.—Schematic view of the passage of an antibody molecule from an sc injection site into the lymphatics through clefts in the endothelium of the terminal lymphatic capillary. In the 1st node encountered, or in more distant nodes of the chain, antibody may bind to normal cells, such as the macrophages (M) and lymphocytes (L), or to tumor cells (T). Antibody not removed from the lymph flow finally passes into the bloodstream, largely via the thoracic duct (modified from ref 14).

When injected sc, molecules the size of IgG (150,000 daltons) or IgM (900,000 daltons) do not pass readily into the local blood capillaries. They are prevented from doing so by the tight vascular endothelium and also by the presence of a continuous basement membrane. Instead, the macromolecules pass into terminal lymphatic capillaries (fig 1), which do not have continuous basement membranes and have endothelial lining that contains clefts between cells (13). The clefts open and close in response to hydrostatic pressure differences and activity of nearby muscles. Lymph flows from the terminal capillaries into vessels of increasing diameter, propelled by a combination of extrinsic muscular action, contraction of smooth muscle in the walls of the vessels, and hydrostatic pressure gradients. Retrograde flow is prevented by a system of one-way valves. Upon reaching a regional lymph node, the lymph first empties into a sinus located beneath the external capsule of the node. It then passes through a series of sinusoids into the medulla and out through an efferent vessel. Within the node, antibody may bind to normal or abnormal cells (14). If not so bound, it passes to more distant nodes in the chain. If still not removed from the lymph flow, it passes into the bloodstream, principally via the thoracic duct. Thereafter, it would be expected to distribute to the various organs as though injected iv in the first place. The lymphatics form a single-pass system filtered by lymph nodes, not a true "circulation" in the sense of the blood vascular system.

There are several clinically interesting targets for lymphatic administration of antibody: lymphomas, lymph node metastases of solid tumors, infectious agents, and also the various lymph node cells involved in the immune response. Some of the possibilities for use against cancers will be considered later.

Polyclonal Antisera in the Lymphatics

In 1975, Order and co-workers (15) reported a case study in which partially purified heteroantiserum directed against ferritin was injected sc in a patient with Hodgkin's disease for localization of regional lymph nodes. In 1980, DeLand and colleagues (16-18) reported a series of patients injected with heteroantiserum against carcinoembryonic antigen for imaging of lymph nodes. In each study, visualization of cancerous nodes appeared to be possible. However, the clinical setting made it difficult to do direct specificity controls, and the correlation with histology was only partial (19).

Monoclonal Antibodies Against Normal Cell Types in the Lymph Node

Initial experiments in our laboratory (14) were done with radioiodine-labeled and fluorescence-labeled antibodies directed against determinants on *normal* cell types of the mouse lymph node: T-cell antigens (Lyt 2, Thy 1.2) and histocompatibility antigens (H-2K^k, H-2D^b). The use of monoclonal antibodies to normal cells made it possible to study the pharmacokinetics in a reproducible, quantitatively interpretable system before progressing to the complexities and biologic heterogeneity of a metastatic tumor. When ^{125}I-labeled anti-H-2K^k IgG was injected in the hind footpads of mice expressing the K^k antigen (fig 2), 10%-20% of the injected dose could be found in the ipsilateral popliteal node 2 hours later. In H-2K^k-negative mice there was 50-fold less popliteal uptake. As indicated in figure 3, popliteal and lumbar nodes were easily imaged under a gamma camera within a few minutes after injection. In other studies, nodes were visualized with as little as 0.25 μCi of ^{125}I. Node images improved over the first few hours before slowly fading. Similar results were obtained for F(ab')$_2$ fragments of the antibody. Organ images were positively identified by the experiment shown in figure 4; the animal was injected (in only one footpad), killed 160 minutes later, and imaged repeatedly as organs were removed one by one.

The anti-K^k antibody was subjected to an extensive characterization of its equilibrium and kinetic binding properties in vitro at 4 °C (20) and at 37 °C (Steller MA, et al, manuscript in preparation). The results were then integrated with those from biodistribution studies in formulating pharmacokinetic models of the delivery process (21). Figure 5 shows one such biodistribution study, that in which the dose of antibody was varied. Antibody appeared to saturate binding sites in the popliteal nodes and then in the lumbar nodes. At a dose of about 1 μg, antibody finally overflowed into the bloodstream and distributed to the liver (also the spleen, kidneys, lungs, etc, not shown). Clearly, the best dose for maximizing the ratio of node image to body background would be about 0.3-0.7 μg in this particular case. It is not yet known whether the same principles will apply in clinical situations with human tumors, but the possibility of such sequential saturation should be considered. These studies provide a basis for imaging of radiolabeled monoclonals directed against normal cell types in human nodes as an alternative to lymphoscintigraphy with ^{99m}Technetium colloids.

Monoclonal Antibodies Against Tumor in the Lymph Nodes

The findings in the normal system were also used in the design of experiments on lymphatic delivery of monoclonals to a metastatic tumor, line 10 (L10) hepatocarcinoma of guinea pigs (22). L10 is a methylcholanthrene-induced, transplantable tumor which metastasizes to regional lymph nodes and to visceral organs. When implanted intradermally in the flank of the animal, it metastasizes first to the ipsilateral superficial distal axillary (SDA) node. D3 is a murine monoclonal IgG$_1$ directed against a 290,000-dalton antigen on L10 cells in both primary tumors and metastases. In vivo it has been shown to accumulate selectively in L10 tumor after iv administration (23,24). D3 was delivered via the lymphatics to the SDA node either from the ipsilateral front paw or from an sc injection site in the flank near the tumor.

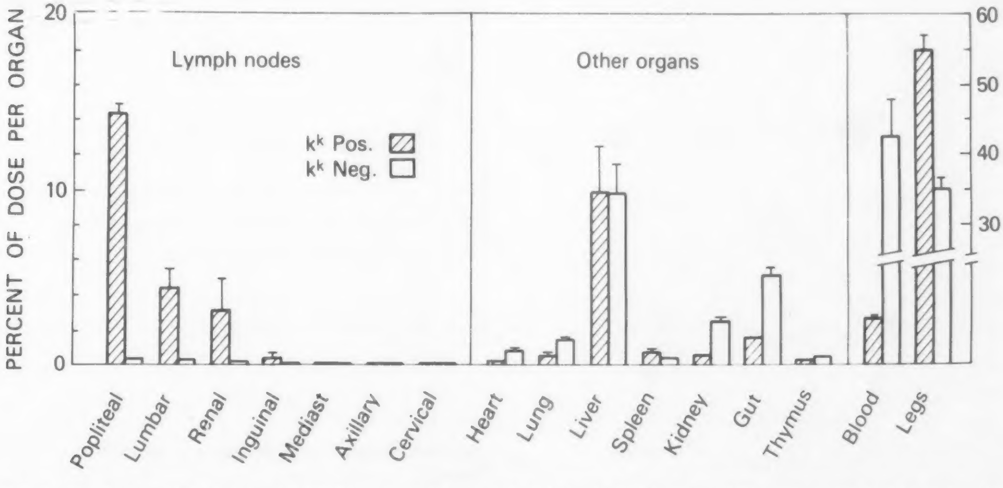


FIGURE 2.—Distribution of ^{125}I-labeled anti-H-2K^k 2 hrs after sc injection in both footpads of K^k-positive and K^k-negative mice. Each footpad was injected with 50 μl of a solution containing 2.7 pmols of protein A-purified antibody (specific activity, 8.3 × 10^{14} dpm/mmol). Bars represent mean values for groups of 3 mice, with standard errors indicated where large enough to show. Sodium dodecyl sulfate gel electrophoresis indicated that most of the ^{125}I counts represented intact IgG. Given the difference in weights between popliteal nodes and liver, the ratio of ^{125}I concentrations in the 2 was approximately 2000:1. From ref 14.

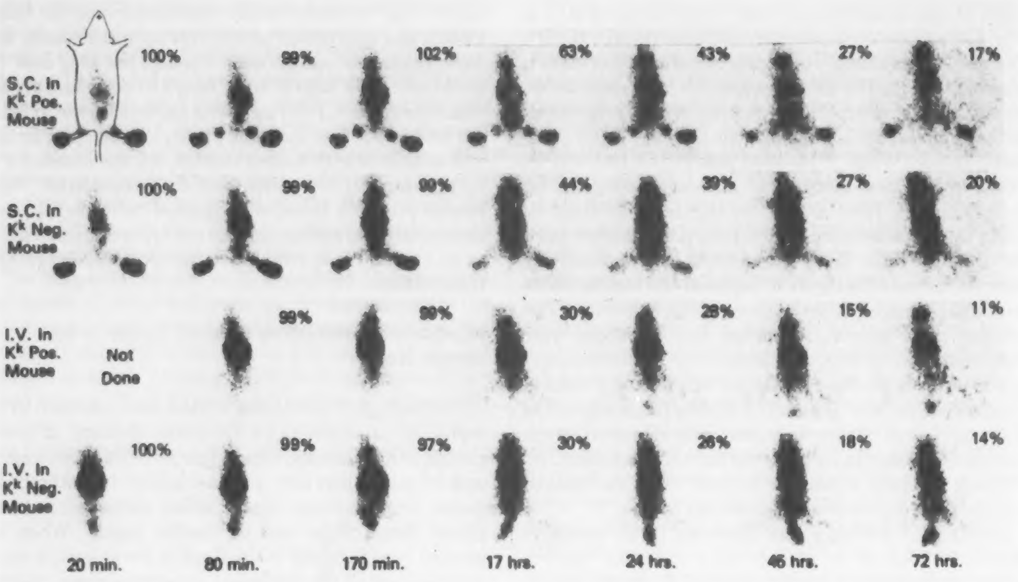

S.C. In
K^k Pos.
Mouse
100%
99%
102%
63%
43%
27%
17%
S.C. In
K^k Neg.
Mouse
100%
99%
99%
44%
39%
27%
20%
I.V. In
K^k Pos.
Mouse
Not
Done
99%
99%
30%
28%
15%
11%
I.V. In
K^k Neg.
Mouse
100%
99%
97%
30%
26%
18%
14%
20 min.
80 min.
170 min.
17 hrs.
24 hrs.
46 hrs.
72 hrs.

FIGURE 3.—Scintigraphic images of H-2K^k-positive and -negative mice injected sc in the hind footpads or iv with ^{125}I-labeled anti-H-2K^k IgG (0.2 μg, 4 μCi/foot). The most prominent spots in the left-uppermost panel are the injection sites. Also prominent as early as 20 mins after injection are popliteal and lumbar nodes, as well as the early development of a body pool. The node images continue to develop over at least the first 150 mins and have begun to fade by 17 hrs. At 17 hrs for whole IgG, the bladder and thyroid are prominent, presumably because of released iodine. Node images are dim for K^k-negative animals injected sc. No node images are seen after iv injection. Diffuse spots over the tail represent small amounts of extravasation from the tail-vein injection site. Each image represents 15,000 counts recorded with a Raytheon Anger gamma camera. Percentages represent counts remaining in the body, as calculated by integration over the body image.

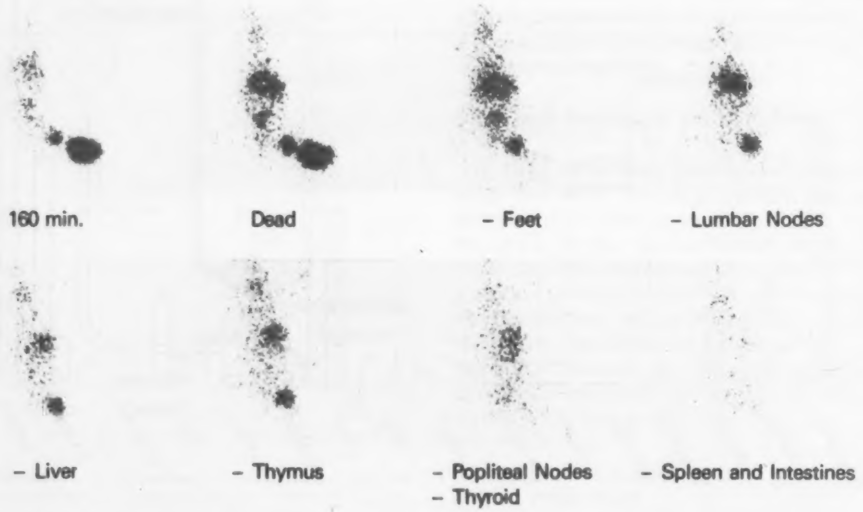

160 min.
Dead
- Feet
- Lumbar Nodes
- Liver
- Thymus
- Popliteal Nodes
- Thyroid
- Spleen and Intestines

FIGURE 4.—Experiment demonstrating the correspondence between images and organs. An H-2K^k-positive mouse was injected in 1 footpad, then killed at 160 mins. Organs were removed one by one, while a sequence of images was being recorded. Considerable Nos. of counts were registered from footpads, popliteal and lumbar nodes, liver/spleen, and thyroid. Very few counts remained in the carcass after removal of the organs noted.

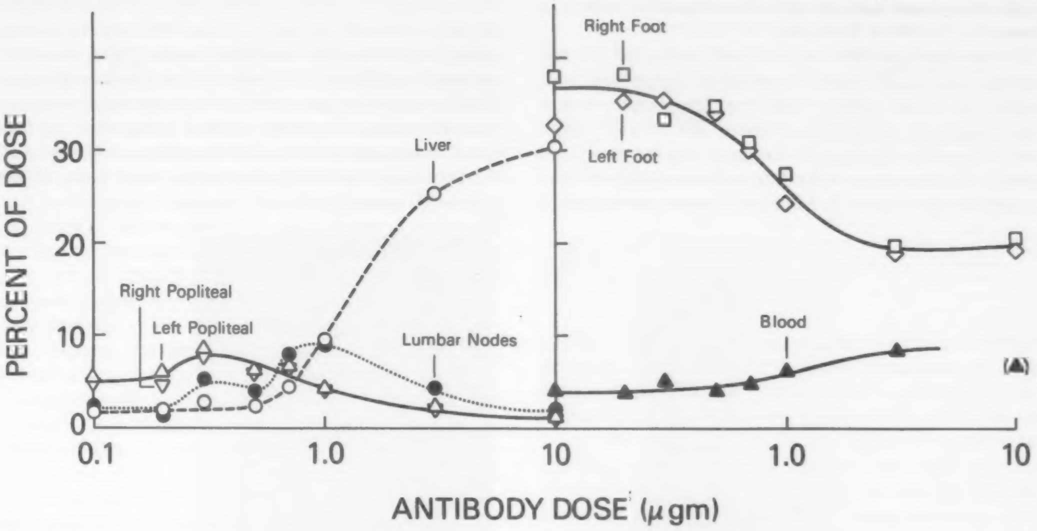


FIGURE 5.—Concentration dependence of ^{125}I-anti-K^k distribution 2 hrs after injection in the hind footpads of K^k-positive mice. The injection volume was 50 μl, and there were 4 animals/group. The liver (and other body organs) increase dramatically in uptake at doses above about 0.8 μg.

Figure 6 shows gamma camera images (22) which indicated localization of radiolabeled D3 in cancerous SDA nodes after sc injection. Dissection and counting confirmed that the observed images represented the lymph nodes. Specificity of binding was demonstrated by double-label experiments with ^{125}I-labeled D3 and ^{131}I-labeled MOPC 21, a murine IgG$_1$ with no known binding specificity. The specific/nonspecific ratio within the lymph node metastasis was on the order of 100:1. There was very little uptake of antibody in cancerous nodes contralateral to the site of injection, indicating that delivery to ipsilateral nodes had taken place principally via lymphatic vessels, not by way of the bloodstream. Iv injection delivered only one-fifth as much D3 to the lymph

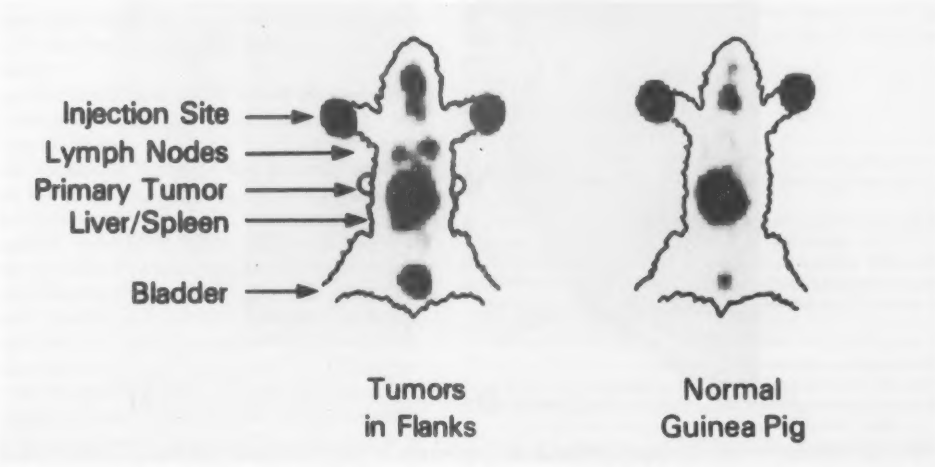


FIGURE 6.—Gamma camera images of guinea pigs 2½ hrs after sc injection in both front feet with ^{125}I-labeled D3 antibody. Left: Day 14 after bilateral inoculation with 10^6 L10 tumor cells. Right: Normal animal. 40,000 counts were recorded with an Anger gamma camera using a low-energy, parallel-hole collimator. Images were smoothed and spontaneous background counts were subtracted. Blood pool background was not subtracted. Dose/foot: 3.5 μCi in 200 μl of phosphate-buffered saline with 1 mg/ml of bovine serum albumin. From ref 22.

node metastases, and the specific/nonspecific ratio was lower than for the sc injection.

It remained possible that antibody delivered via the lymphatics was not bound to metastatic tumor itself but, rather, to tumor antigen shed from the primary tumor into lymphatic vessels and filtered out by the lymph nodes. However, localization in tumor was demonstrated directly by autoradiography (22), as shown in figure 7. At an early stage of growth, metastatic tumor spreads along the subcapsular space, before infiltrating the parenchyma of the node. Areas of radioactivity shown in autoradiographs from sectioned SDA nodes (fig 7, a′ and b′) corresponded closely to the histologic location of tumor (fig 7, a and b). At a later stage of growth, the metastases formed masses of several hundred milligrams. At that stage, antibodies still reached the tumor and labeled it, but it was not clear whether all, or only part, of the tumor was accessible.

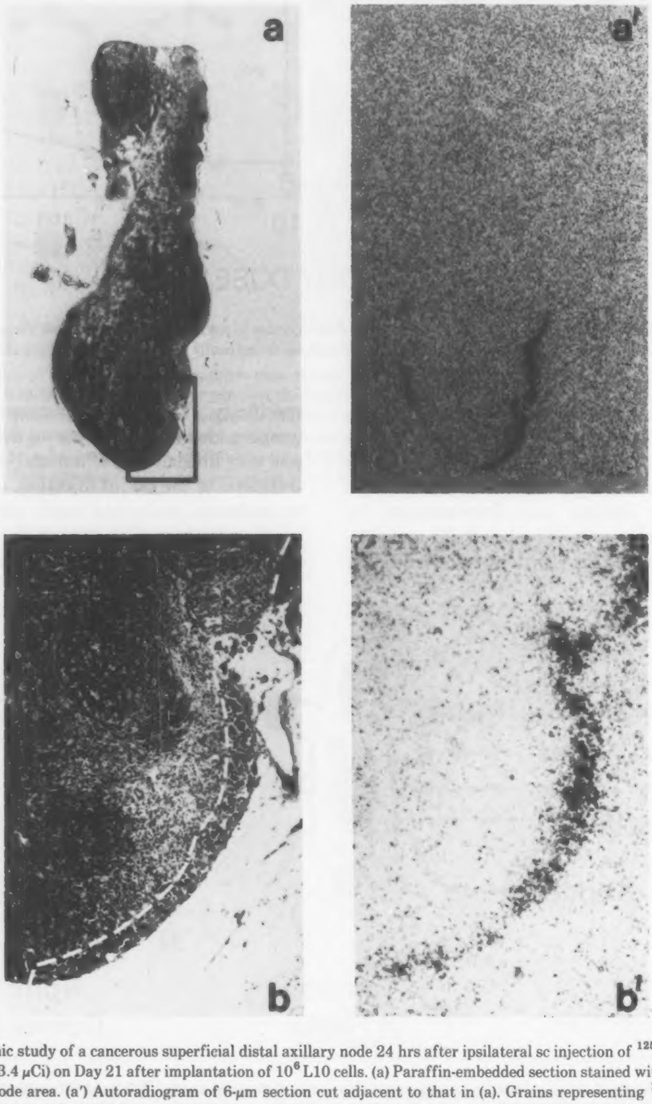


FIGURE 7.—Autoradiographic study of a cancerous superficial distal axillary node 24 hrs after ipsilateral sc injection of ^{125}I-D3 IgG. The guinea pig was injected with 34 μg of D3 (3.4 μCi) on Day 21 after implantation of 10^6 L10 cells. (a) Paraffin-embedded section stained with hematoxylin and eosin. Tumor occupied 2% of the node area. (a′) Autoradiogram of 6-μm section cut adjacent to that in (a). Grains representing ^{125}I-labeled antibody are seen along the rim of the node at one end. (b) Higher magnification corresponding to the inset box in (a). Large, pleiomorphic tumor cells occupy the area demarcated by white dashed line. (b′) Autoradiogram of section adjacent to that in (b). Magnification of (a) and (a′), × 14, of (b) and (b′), × 57. From ref 22.

DISCUSSION

Since there are as yet no clinical data on lymphatic delivery of monoclonal antibodies, the feasibility of such an approach in humans can be inferred only from the animal studies. As shown in table 1, lymphatic delivery has several possible advantages over the iv route for reaching regional lymph nodes. Much lower doses can apparently be used for diagnostic imaging of smaller masses. The popliteal nodes of mice weighed < 1 mg, but they were imaged within a few minutes using < 1 μCi of radioiodine (14). As little as 2 mg of early metastatic tumor in the guinea pig was also imaged, though not as soon after injection (22). Of importance to therapy, the lower dose would presumably decrease systemic toxicity, especially with antibodies bearing drugs, toxins, or radionuclides.

Perhaps most interesting, localization in the lymphatics should reduce binding of the antibody to cross-reactive antigen on normal cells. The studies in mice were done with antibody directed against major histocompatibility antigens expressed on most cells in the animal. That system represented the logical extreme of "cross-reactive" binding, but localization in the regional lymph nodes was still profound. Only at doses which saturated the binding capacity of the nodes and allowed overflow into the systemic circulation did antibody bind significantly to the H-2 antigen expressed on other organs (Steller MA, et al, manuscript in preparation). Many monoclonal antibodies derived by immunization with tumor cells are not highly specific and have been ruled out for clinical use by the iv route because of cross-reaction. Antibodies to breast and colon tumors may, for example, bind to the normal tissues. Lymphatic use of those antibodies would not be precluded, except perhaps if the antigen were expressed on normal cell types in the lymphatics or shed in large amounts into the nodes from primary tumor.

If, on the other hand, an antibody were highly selective for tumor and able to circulate for long periods in the blood, then multiple passes through vessels supplying the node would eventually saturate the binding sites of tumor cells there. In such a case, lymphatic delivery would be advantageous only if speed were required.

The lymphatic route is, of course, limited to regional lymph nodes; it cannot be used to detect or treat metastases in such common sites as liver, lung, and brain. One can, however, identify several clinical settings in which the approach may be useful. Assessment of lymph node status is currently important in the staging of such malignancies as Hodgkin's disease, malignant melanoma, and carcinomas of lung, colon, breast, testis, prostate, and cervix. If appropriately optimized through studies in animals and in the clinical setting, the lymphatic approach may in the end be the most sensitive noninvasive technique available for identifying occult lymph node metastases. Early metastases, such as those imaged in the guinea pig via the lymphatics, were too small to be found clinically or visualized by current techniques of computerized tomography or standard radiology (25). Information on lymph node status can be used, for example, in optimizing irradiation fields (as in testicular and cervical carcinomas) or for decisions about adjuvant therapy (as with breast cancer). The studies cited indicate that antibodies are well-mobilized from the tissue space after simple sc administration. However, they could if necessary be injected into cannulated lymphatic vessels, as are radio-opaque materials for lymphangiography.

TABLE 1.—Comparison of sc and iv administration of monoclonal antibodies for delivery to lymph nodes

Possible limitations of sc administration	Possible advantages of sc administration
Restriction to regional nodes	Lower dose
Lymph vessels must be patent	Detection of smaller metastases
Possible toxicity at injection site	Faster detection of tumor
	Less systemic toxicity
	Less competition by circulating antigen
	Less cross-reaction with antigen expressed on normal cells

The therapeutic potential of monoclonal antibodies in the lymphatics will be easier to assess once there has been significant clinical experience in the diagnostic mode. However, current conventional therapy indicates some of the options. Irradiation of regional nodes may improve the prognosis for selected patients with tumors which disseminate early via the lymphatics, for example, in some cases of Hodgkin's disease, non-Hodgkin's lymphoma, and seminomatous testicular carcinoma. Antibodies delivered via the lymphatics may prove to be a useful form of adjuvant therapy for occult lymph node metastases.

REFERENCES

1. VON BEHRING E. Untersuchungen uber das Zustandekommen der Diphtherie-Immunitat bei Theiren. Dtsch Med Wochenschr 16:1145-1148, 1890.
2. HERICOURT J, and RICHET C. De la sérothérapie dans le traitement du cancer. C R Seances Acad Sci 121:567-569, 1895.
3. EHRLICH P. Collected Studies on Immunity. New York, Wiley, 1906.
4. WITEBSKY E. Disponibilitat, und Spezifitat alkohollöslicher Strukturen von Organen und Bosartigen geschwulsten. Z Immunitaetsforsch 62:35-73, 1929.
5. HIRZFELD L, HALBER W, and LASKOWSKI J. Untersuchungen uber die serologischen Eigenschaften der Gewebe; uber serologische Eigenschaften der Neubildungen. Z Immunitaetsforsch 64:81-113, 1929.
6. PRESSMAN D, and KORNGOLD L. The in vivo localization of anti-Wagner osteogenic sarcoma antibodies. Cancer 6:619-623, 1953.
7. BALE WF, SPAR IL, and GOODLAND RL. Experimental radiation therapy of tumors with I-131-carrying antibodies to fibrin. Cancer Res 1488-1494, 1960.
8. DECARVALHO S, and RAND HJ. Effects of iso- and hetero-antibodies against specific antigens of the transplantable Novikoff hepatoma of the rat (solid form). Nature 193:950-952, 1962.

9. GHOSE T, NORVELL ST, GUCLU A, ET AL. Immunotherapy of cancer with chlorambucil-carrying antibody. Br Med J 3:495-499, 1972.
10. ORDER SE, DONAHUE V, and KNAPP R. Immunotherapy of ovarian carcinoma: an experimental model. Cancer 32:573-579, 1973.
11. GOLDENBERG DM, PRESTON DF, PRIMUS FJ, ET AL. Photoscan localization of GW-39 tumors in hamsters using radiolabeled anti-carcinoembryonic antigen immunoglobulin G. Cancer Res 34:1-9, 1974.
12. KOHLER G, and MILSTEIN C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495-497, 1975.
13. LEAK LV. Studies on the permeability of lymphatic capillaries. J Cell Biol 50:300-323, 1971.
14. WEINSTEIN JN, PARKER RJ, KEENAN AM, ET AL. Monoclonal antibodies in the lymphatics: toward the diagnosis and therapy of tumor metastases. Science 218:1334-1337, 1982.
15. ORDER SE, BLOOMER WD, JONES AG, ET AL. Radionuclide immunoglobulin lymphangiography: a case report. Cancer 35:1487-1492, 1975.
16. DELAND FH, KIM EE, CORGAN RL, ET AL. Axillary lymphoscintigraphy by radioimmunodetection of carcinoembryonic antigen in breast cancer. J Nucl Med 20:1243-1250, 1979.
17. DELAND FH, KIM EE, and GOLDENBERG DM. Lymphoscintigraphy with radionuclide-labeled antibodies to carcinoembryonic antigen. Cancer Res 40:2997-3000, 1980.
18. ______. Reply to letter by Ege, ref 19. J Nucl Med 21:205-207, 1980.
19. EGE GN, and BRONSKILL MJ. Lymphoscintigraphy with antibodies to CEA. J Nucl Med 21:805-806, 1980.
20. DOWER SK, OZATO K, SACHS DH, ET AL. The interaction of monoclonal antibodies with MHC class I antigens on mouse spleen cells. I. Analysis of mechanism of binding. J Immunol, in press.
21. WEINSTEIN JN, STELLER MA, COVELL DG, ET AL. Use of monoclonal antibodies for diagnosis and therapy of tumor metastases in lymph nodes. *In* Proceedings of the Fifth International Symposium on Affinity Chromatography and Biological Recognition. In press.
22. WEINSTEIN JN, STELLER MA, KEENAN AM, ET AL. Monoclonal antibodies in the lymphatics: selective delivery to lymph node metastases of a solid tumor. Science 222:423-426, 1983.
23. KEY ME, BERNHARD MI, HOYER LC, ET AL. Guinea pig line 10 hepatocarcinoma model for monoclonal antibody serotherapy II. In vivo localization of a monoclonal antibody in normal and malignant tissues. J Immunol 130:1451-1457, 1983.
24. BERNHARD MI, HWANG KM, FOON KA, ET AL. Localization of ^{111}Indium and ^{131}Iodine labeled monoclonal antibody in guinea pigs bearing line 10 hepatocarcinoma tumors. Cancer Res 43:4429-4433, 1983.
25. MOAK GD, COCKERILL EM, FARBER MO, ET AL. Computed tomography vs standard radiology in the evaluation of mediastinal adenopathy. Chest 82:69-75, 1982.

Role of Receptors in Mediating Steroid Hormone Effects in Human Breast Cancer

Marc E. Lippman* and Attan Kasid[1]

The purpose of this brief review is to provide current information concerning molecular aspects of endocrine regulation of human breast cancer. This is a rapidly moving and complex field and, of necessity, we have been forced to be highly selective and to allow (hopefully, not too frequently) our own prejudices and paradigms to influence our selection. We will attempt to trace the course of hormone and antihormone into the cell to their eventual sites of interaction with specific genes.

Receptors

In recent years, substantial evidence has suggested that steroid hormones exert their effects on target cells primarily at the level of gene transcription (1-5). Although the ultimate influence of these hormones seems to be the induction of specific proteins, the basic questions remain as to how these steroid hormones regulate specific genes and gene products and how the target tissue specificity of their action is maintained. Jensen and Jacobson (6) were the first to propose the existence of a tissue-specific cytoplasmic protein, called the receptor, that could selectively bind the steroid hormone, eventually resulting in its accumulation at the target site. Each receptor binds its respective hormone with high affinity and great specificity. Receptors for steroid hormones have been identified in a wide variety of target cells for estrogens (7,8), progestins (9), glucocorticoids (10,11), androgens (12,13), aldosterone (14), and 1α,25-dihydroxyvitamin D (12,13). Similar receptors apparently exist for plant and insect steroids, as well (15). How the receptor molecule transfers regulatory information from the steroid hormone to the target gene is not fully understood. According to general consensus, after the hormone binds selectively to its cytosol receptor, the complex undergoes some kind of primary structural or conformational change, alternatively referred to as activation or transformation, which increases the binding affinity of the complex for nuclear components. An excellent comprehensive review that highlights a general model of receptor structure and function has been recently published by Grody and co-workers (16). Some data also suggest that transformation may be an intranuclear event (17,18). Inside the nucleus, the transformed hormone-receptor complex is thought to interact with genomic sites, either directly or following a primary interaction with chromatin "acceptor" proteins to initiate the induction of metabolic alterations.

In unraveling certain facts about estrogen action, human breast cancer cell lines are an attractive model for the mechanistic study of hormone-mediated gene expression. This system, which is characterized by a high degree of viability and unlimited growth potential, permits the study of hormonal effects on a homogeneous cell population which is uniformly exposed to a variety of experimental conditions. The study of the direct effects of individual hormones on neoplastic human tissue can be measured and isolated from secondary endocrine effects in these cells.

The two cell lines most commonly employed in these studies are MCF-7 and ZR-75-1. Ample documentation of their human and mammary nature has been provided (19-24). Both cell lines possess specific, high-affinity saturable cytosol binding for estradiol, demonstrated by Scatchard analyses of the competitive binding information and by sucrose density gradient ultracentrifugation analyses (25-29). In additon, they possess specific receptors for progestins, androgens, glucocorticoids, triiodothyronines, vitamin D, calcitonin, retinoids, insulin, EGF, and prolactin (30,31). Therefore, these cell lines are useful in investigating questions of hormonal regulation of breast cancer growth. Many of the specific effects regulated by estrogens and antiestrogens are summarized in table 1.

MCF-7 cells contain 60-100 fmols of cytosol estrogen receptor per milligram of cytosol protein, with a dissociation constant for estradiol ranging from 0.06 to 2.5 nM, depending on assay conditions. The ZR-75-1 cells possess approximately 30 fmols of cytosol receptor per milligram of cytosol protein, with a similar dissociation constant as

---

[1] Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Marc E. Lippman, MD, Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 12N226, National Institutes of Health, Bethesda, MD 20205.

TABLE 1.—Responses of breast cancer cells to estrogens

Growth responses	DNA synthesis	Inducible proteins
Cell No. (refs 12-15, 27-29,41,46-48,77)	^{3}H-thymidine incorporation (refs 12-15)	Progesterone receptor (ref 55)
Cloning efficiency	Thymidine kinase (ref 37)	Lactic dehydrogenase isoenzymes (ref 61)
Amino acid incorporation (refs 12-15)	Thymidylate synthetase (ref 58)	Plasminogen activator (ref 62)
Morphologic changes (ref 76)	Carbamyl phosphate synthetase (ref 58)	24K protein (refs 17, 63,64)
	Dihydrofolate reductase (ref 59)	52K protein (refs 46,56)
	Aspartate transcarbamylase (ref 58)	
	DNA polymerase (ref 49)	
	Mitotic index (ref 60)	

in MCF-7 cells. In our laboratory, estrogen receptor from MCF-7 cells has been partially purified (1160-fold over cytosol) by a combination of methods, including ammonium sulfate precipitation, affinity chromatography, and sephadex G-100 gel filtration (32). The partially purified estrogen-receptor fragment sediments around 4S and has a molecular weight of about 50,000, which is identical to that obtained for crude cytosolic receptor.

Zava and McGuire have demonstrated that MCF-7 cells are characterized by high concentrations of unoccupied estrogen receptors, both in the cytosolic and nuclear fraction. The receptors for androgens and progestins, on the other hand, are apparently localized in the cytosol. More recently, unoccupied nuclear estrogen sites have been proposed in human endometrium and myometrium, pig uteri, and immature rat uteri (34-37). In rat uterus, the number of such sites is small (38). In the absence of estrogen, the cytosol of confluent cells contains 0.5 pmol of free receptor per milligram of DNA, while a nuclear KCl extract contains 1.2-1.5 pmols of unoccupied receptor per milligram of cellular DNA. In rapidly growing cells, this level of unoccupied sites has been reported to increase to 0.8 and 5 pmols per milligram of DNA for cytosol and nuclear fractions, respectively. These unoccupied sites have high-affinity binding for estradiol and possess most of the characteristics attributed to estrogen receptor. Both cytosol and nuclear unoccupied receptors sediment at 4S in high-salt sucrose gradients. However, the cytosol receptor sediments at 9S in low-salt, but the nuclear receptor sediments at about 4S-5S when diluted to low-salt conditions (33). When MCF-7 cells are grown in estrogen-free medium, there are no filled nuclear sites. Within minutes of estadiol addition, the cytosol and nuclear unoccupied sites are no longer measurable and all the cellular receptor appears in the nucleus bound to estradiol. The concept of an unusual intracellular distribution of estrogen receptor is controversial (39-41); furthermore, whether activation of receptor occurs in cytoplasm or nucleus may be moot, since receptor in the absense of hormone appears to have little biologic activity.

Receptor Activation and Transformation

Most studies with steroid hormone receptor systems indicate that nuclear translocation and occupation of chromatin binding sites, as well as DNA binding, are dependent on some prior structural change in the hormone-receptor complex. A variety of factors including salt, temperature, dilution, nucleotides, and, in some cases, the ligand appear to bring about the requisite changes in vitro. For some receptors, this transformation is associated with major apparent changes in molecular weight, sedimentation properties, isoelectric point, quaternary structure, and other detectable physical alterations. This transformation event may result in exposing positively charged residues on the surface of the receptor, thus increasing its affinity for DNA-cellulose, phosphocellulose, ATP-sepharose, and other polyanions (42-45). However, the molecular basis of these changes is not known and there is no direct proof to show that these in vitro changes are essential to the action of steroid hormones. Mechanistically, it appears likely that estrogen receptor transformation involves estradiol-induced receptor dimerization or assocation with a nonsteroid binding subunit, with the result that the 4S form (molecular weight, $\sim$ 80,000) of receptor (calf uterus) is converted to a 5S form ($\sim$ 130,000). Other data suggest that dimerization is between two equal subunits (45). This shift obviously implies a molecuar weight of $\sim$ 50,000 for the added subunit, and it appears that the binding of a nonreceptor 50K protein alters the conformation of the 80K receptor to a more nuclear-binding form (46). Furthermore, there are suggestive reports implicating a low molecular weight inhibitor (which can be removed by simple dilution or dialysis or by gel filtration) of estrogen-receptor transformation in rat uterus (47). A similar inhibitor, which is heat-stable, has been claimed for the glucocorticoid receptor by Bailly and co-workers (48). Similarly, Simons et al (49) have reported the presence of a macromolecular inhibitor in the cytosol of rat hepatoma HTC cells, which binds to the glucocorticoid-receptor complex and inhibits receptor binding to nuclei, chromatin, and DNA. Another proposed mechanism of transformation, involving receptor proteolysis, was reported by Puca et al (50), who identified a Ca^{++}-activated receptor-transforming protease activity in calf uterine cytosol. This protease transformed 5.3S estrogen receptor to a nuclear-binding 4.5S protein. However, it is difficult to reconcile these results into a general mechanism, especially in light of the observation by Andre and co-workers (51) that lamb uterine receptor transformed by this protease activity does not resemble the nuclease-extracted nuclear receptor. Additional reports have noted that sodium molybdate, a potent phosphatase inhibitor, stabilizes glucocorticoid receptor (52,53), progesterone receptor (54), and rat uterine estrogen receptor (55) in association with inhibition of receptor transformation. This suggests that receptor transformation may involve a specific dephosphorylation mechanism. However, to date, there is no direct evidence demonstrating that such a process involves the receptor itself. Whether or not the hormone receptor pathway involves a phosphorylation-dephosphorylation process, it now appears that molybdate may act directly on the receptor molecule itself. While these analyses and interpretations are interesting, transformation may be purely an in vitro phenomenon, brought about by coincidental artifacts resulting from cell disruption. What the true native form of receptor is and what precise changes in receptor lead to the ability to cause genomic activation are questions which remain to be answered.

We have performed extensive studies in our laboratory aimed at understanding the dynamics of this estrogen-receptor activation in MCF-7 cells (56,57). Using DNA-cellulose binding as a measure of receptor transformation, we find that temperature elevation does not efficiently activate the estrogen-receptor complex in this system; however, progesterone, glucocorticoid, and androgen receptors were activated by these means. Ribonucleotides activate all four of these receptors. John and Moudgil (58) found that 5-10 mM ATP enhanced binding of rat liver glucocorticoid receptor to nuclei at 4°C; this ability to bind nuclei was comparable to that obtained by warming cytosol to 23°C. Although there is no evidence that ATP interacts directly with the receptor, their studies with various RNA and DNA polymerase inhibitors (rifamycin AF/013, aurintricarboxylic acid) suggest that ATP-mediated stimulation is the result of an allosteric transformation which occurs when ATP combines with a specific nucleotide-binding site on the receptor (59). Sodium molybdate can partially block ATP-induced activation of estrogen receptor in MCF-7 cells (57). Interestingly, exogenous ribonuclease can increase binding of estrogen- and glucocorticoid-receptor complexes from MCF-7 cells to DNA-cellulose (57). In fact, purified RNA extracted from MCF-7 cells can inhibit estrogen and glucocorticoid binding to DNA-cellulose and, surprisingly, in the same study, there was evidence consistent with an association of RNA with estrogen-receptor complexes from MCF-7 cells under low-salt conditions. This association of steroid-receptor complex with RNA accompanied by increased binding to DNA-cellulose after RNase treatment raises the possibility that activation of estrogen-receptor complex in this system may involve the dissociation of RNA. Possibly, a loosely associated piece of RNA would otherwise conceal a site on the receptor with a high affinity for DNA. Recently, Liao et al (60) have shown that poly (U), poly (G), poly (X), poly (I), rRNA, tRNA, and poly (A)-RNA all promote the release of androgens and other steroid hormone-receptor complexes that were bound to DNA. Further studies on the interaction between steroid hormone-receptor complexes, RNA, and ribonucleotides may reveal more about this interesting interaction.

Receptor Processing

Following translocation of the hormone-receptor complex to the nucleus in MCF-7 cells, Horwitz and McGuire (61-64) described a time-dependent decline in nuclear estradiol-binding activity, such that within 3-5 hours, only 30% of the sites were measurable. This specific event, which is not accompanied by reappearance of receptor in the cytoplasm, was termed "processing." Processing was essentially complete by 5 hours, after which time the nuclear-receptor complex stabilized at a new steady-state level. Receptor processing in MCF-7 cells correlates temporally with estrogen-induced progesterone-receptor synthesis. When MCF-7 cells are exposed to antiestrogens, processing events are different. Receptor decrease was either diminished in the presence of tamoxifen or failed to occur entirely when nafoxidine was added to cell cultures. Moreover, when added with hormone, dactinomycin completely inhibited the normal processing of estradiol-bound nuclear receptors. Interestingly, except for chromomycin A3, several other inhibitors of cell function, which likewise intercalate into DNA and inhibit RNA transcription, failed to prevent processing, suggesting that dactinomycin action may be direct rather than involving RNA and protein synthesis (63,64).

The mechanism and significance of this important intranuclear event are not known. We have undertaken an investigation of the mechanism of this possible regulatory event in MCF-7 cells. We have studied both the quantitative and qualitative changes in the nuclear receptor following its translocation, that appear to be coupled with tighter binding affinity of the receptor with the genomic sites. Our data suggest that estrogen-binding activity in MCF-7 cells is not an absolute number; instead, it is strongly dependent on the proliferative state of the cell, as well as a variety of other factors. This appears to be compatible with several reports in the literature (65,66), suggesting that receptor concentration is cell-cycle-dependent. Our results suggest that cells grown in the presence of insulin or medium conditioned by other MCF-7 cells have more proliferative activity, which is accompanied by at least three times less receptor content (~ 30,000-50,000 sites/cell) versus cells grown in the absence of insulin or conditioned medium (~ 100,000 sites/cell). The latter manipulation, in particular, appears to enhance the proliferative activity of MCF-7 cells and is accompanied by a down regulation of estrogen-receptor activity. We find that exposure of cells grown in the absence of insulin or conditioned medium to estradiol resulted in a greater extent of receptor loss (~ 60%) at 6 hours when binding was measured in intact cells or nuclear KCl extracts. These findings are compatible with the quantitative loss in competible binding during "processing," described by Horwitz and McGuire (61-64). However, in studies performed with MCF-7 cells grown in the presence of 5×10^{-7} M insulin or conditioned medium, we find both by Scatchard analyses of whole-cell-binding assays and competible binding in salt-extractable nuclear fractions that the time-dependent loss in nuclear-receptor activity at 6 hours is much smaller and may even be completely eliminated. This strongly suggests that following continued exposure to estradiol, quantitative loss of measurable estrogen-receptor binding is variable. What precisely controls this loss is still unknown; however, the important issue we chose to address was whether this quantitative loss in receptor binding was critically required for hormone action in these cells. To answer this, we examined estrogen-induced progesterone-receptor induction in MCF-7 cells, growing either in the absence of conditioned medium (high receptor activity, with maximum quantitative loss at 6 hours) or in the presence of conditioned medium (low receptor activity, with minimal quantitative loss at 6 hours). Interestingly, we found progesterone-receptor induction to the same extent in both cases, regardless of the degree of quantitative loss in estrogen-receptor binding activity. This evidence argues that quantitative loss is not selectively a prerequisite for hormone action in MCF-7 cells.

On the contrary, we find that following translocation of estrogen receptor to the nucleus, a new, less extractable receptor form appears, which has a variety of altered characteristics. This novel form of nuclear receptor can be physically distinguished by sucrose density gradient analysis. We have consistently found that this "processed" receptor sediments faster (5S) than the freshly translocated receptor (4.4S). Based on experiments with an antireceptor monoclonal antibody ([D547 Spγ], supplied by Dr. G. Greene), we propose that this nuclear transformation occurs to the receptor itself rather than representing transfer of ligand to some alternative species. We believe that this conformational change may represent transition to a more productive location of the estrogen receptor in the nucleus.

Following continued exposure of MCF-7 cells to estradiol, we have also found time-dependent alterations in the binding kinetics of nuclear receptor. Measuring the dissociation of hormones by isotopic exchange from nuclear receptor isolated from directly labeled cells, we have observed that the freshly translocated receptor displayed a biphasic pattern with a half-life of ~ 8.5 and 216 minutes corresponding to the first and second dissociating components, respectively. In contrast, the dissociation of chronically translocated receptor (following direct labeling of cells with estradiol for 5-6 hours) was slow and followed a monophasic pattern with a half-life of ~ 200 minutes.

Finally, a different set of experiments done in our laboratory suggests that transformation of the unprocessed receptor to the processed state is related to another nuclear event, manifested by a true loss of binding, but only of rapidly dissociating estradiol sites with the same time course. Both receptor processing and loss of rapidly dissociating sites follow a similar time course and are inhibited by dactinomycin. Ethidium bromide, which is similar to dactinomycin in that it intercalates in the minor groove of DNA regions rich in G-C base pairs (67) and can affect DNA, RNA, and protein synthesis, alters neither the loss of lower affinity sites nor the receptor processing. This implies some specificity of DNA-estradiol binding sites. It is also possible that the low-affinity rapidly dissociating sites may be similar to nuclear type II sites of rat uterus (68) or competible binding to various other subcellular structures, such as nuclear membranes, nuclear matrix, chromatin, and DNA.

In light of these novel alterations in the estrogen-binding characteristics during nuclear processing, it is our working hypothesis that certain qualitative, rather than quantitative, changes in the nuclear receptor activity may be intimately coupled to tighter and productive binding of the transformed nuclear receptor to the genomic sites.

Inactivation of Estrogen Receptor

Auricchio et al (69) have recently reported that calf and mouse uterine nuclei contain an activity that inactivates both cytosol and nuclear estradiol-receptor complexes. This nuclear-receptor-inactivating activity is increased in the presence of dithiothreitol and inhibited by several phosphatase inhibitors, as well as by phosphatase substrates. The nuclear inactivating enzyme does not inactivate receptor when complexed with antiestrogens. Based on these observations, it is possible that the dephosphorylation of translocated receptor by nuclear phosphatase activity may be an important step in the pathway of estrogen action. The ineffectiveness of the nuclear phosphatase activity in dephosphorylating the antiestrogen-receptor complex may, in turn, explain the prolonged retention of antiestrogen-receptor complexes and the failure to observe receptor processing in MCF-7 cells upon continued exposure to antiestrogens (70). Auricchio et al (71) have also hypothesized that the inactive receptor may be reactivated through phosphorylation by a cytoplasmic ATP-dependent enzyme. A similar possibility that the steroid-free glucocorticoid receptor from mouse fibroblasts and rat liver can be inactivated by dephosphorylation and that only the phosphorylated form of the receptor is capable of binding glucocorticoids has been reported (72,73). These studies suggest that a phosphorylation-dephosphorylation mechanism may be responsible for regulating the level of active receptor in the cell.

Detection of Estrogen-Receptor Complexes: Estrogen-Receptor Antibodies

A considerable number of methods have been developed to qualitatively and quantitatively assay steroid receptors in breast cancer cells. Progress towards development of newer techniques which are rapid, more sensitive, reliable, and economical is encouraging. These assay methods, along with various advantages and difficulties in their performance, have been extensively discussed in recent reviews (31,74-76). Two commonly employed analytical methods for estrogen-receptor measurement include dextran-coated charcoal with Scatchard plots and sucrose gradient ultracentrifugation. Other methods, including gel filtration, ion exchange chromatography, electrophoresis, and isoelectric focusing differ primarily in the procedure used to separate hormone bound to the receptor protein from hormone that remains unbound.

All of these detection characterization and measurement methods are based on the use of radiolabeled hormone as a tag for the receptor protein to which it binds. This ligand-labeling approach, although routinely used for quantitation and following receptor purification procedures, imposes certain restrictions. The labeled steroid may fail to recognize the receptor in its early stage of synthesis or in the later stages in the chain of events where the hormone and receptor may have dissociated. Another difficulty arises when some of the receptor sites in a tumor may be previously occupied by endogenous hormone and thus escape detection, unless some rigorous exchange assay is performed. Since the ligand-hormone interaction is noncovalent, the bound ligand is prone to be displaced during experimental manipulation of receptor preparations. The problem is complicated by the fact that steroids may bind to a number of nonreceptor proteins present as plasma proteins or cellular contaminants in cytosol samples. Moreover, a detailed understanding of the process of receptor turnover, activation, translocation, and gene regulation is far from complete. Therefore, to advance our knowledge of the overall pattern of hormone-receptor interaction, newer methods that do not depend on the binding of labeled steroid need to be developed. One such approach is the use of specific antireceptor antibodies as probes for detecting and studying receptor structure and function and as a reagent for purification of receptor.

In 1977, Greene and colleagues (77,78) prepared antibodies to estrogen receptor (estrophilin) in rabbit, goat, and rat, by immunizing the animals with partially purified calf uterine nuclear estrogen-receptor immunogen. These antireceptor antibodies reacted not only with nuclear and estranuclear estrophilin from calf uterus, but also with cytosolic and nuclear estrogen receptors from reproductive tissues of every animal tested, including human breast cancers and the nonmammalian hen oviduct. Even the meroreceptor (79) of human breast cancer cytosol retains the ability to react with antireceptor antibody generated in rabbit. More recently, Coffer and King (80) have reported preparation of antireceptor antibody in sheep immunized with purified cytosol receptor from normal human myometrium. This antibody cross-reacts with cytoplasmic estrogen receptor from human uterus, breast tumors, MCF-7 cells, and also with rat uterus, rat pituitary, and calf uterus. This antibody did not react either with other steroid receptors or with nonreceptor-estrogen binding proteins. However, contrary to previous reports (77,78), this antireceptor antiserum did not cross-react either with CA^{++}-stabilized or trypsin-digested form of rat uterine receptor or with nuclear (5S) receptor from rat uterus, suggesting loss or unavailability of antigenic determinants from these subunits. The interaction of estrophilin with these antibodies does not require the presence of estrogen, nor does this receptor-antibody interaction inhibit the ability of receptor to bind estradiol. Therefore, in such assays, the labeled steroid can still serve as a convenient marker for antibody-receptor association. Such a complex may be analyzed as a soluble immune-receptor complex on sucrose density gradients or as an insoluble complex when a second antibody raised against the immunoglobulin of the immunized animal is added to estradiol antireceptor-antibody complex. Antireceptor antibodies have also been produced against progesterone receptor from rabbit uterine cytosol (81). The anti-rabbit progesterone receptor antibodies reacted with progesterone receptors from other mammals, including human uteri and breast cancer, but failed to cross-react with a nonmammalian progesterone receptor from chick oviduct.

This approach has been further advanced by development of monoclonal antibodies against estrophilin. These monoclonal antibodies react directly with both occupied and unoccupied estrogen receptors of MCF-7 cytosol and nuclear fractions, and also with estrogen receptors of human breast tumors and certain other mammalian species (82,83). Two of the clones (D547 Spγ and D75 P3μ) secrete rat IgG of the γ2a subclass that react with cytosol and nuclear estrogen receptor to form an 8S immunoreceptor complex in salt-containing sucrose gradients. Another clone (D58 P3γ) secretes IgM that interacts with the cytosol and nuclear estrogen receptor to form immunoreceptor complexes of 18S–19S in high-salt gradients. By growing the clone in the presence of ^{35}S-methionine, radiolabeled monoclonal IgG to human estrogen receptor has been prepared; the antibody can also be iodinated. Recent work has been directed towards optimizing immunoradiometric and enzyme-linked immunochemical determinations of estrogen receptor (84). An immunoperoxidase technique combined with these monoclonal antibodies has been used for immunocytochemical localization of estrogen receptors in breast cancers (84). Similarly, recent findings on the production of antiprogesterone monoclonal antibody, which blocks pregnancy in mice when given as single or multiple injections during preimplantation, should prove invaluable for future studies on fertility control (85)

Antiestrogen-Receptor Complexes

MCF-7 cells present an excellent model for exploration of the biochemical differences in pathways of estrogen and antiestrogen action (28,29,33,86–88). Antiestrogens bind to and interact with the estrogen receptor in MCF-7 cells. In fact, direct binding studies with radiolabeled tamoxifen have shown that the number of tamoxifen and estradiol binding sites is equivalent (89). Tamoxifen competition for MCF-7 cytosol receptor has also been demonstrated in sucrose density gradients and competitive binding assays, using increasing concentrations of tamoxifen to displace ^{3}H-estradiol binding. This, together with the facts that (*a*) antiestrogen inhibition of both growth and macromolecular synthesis is ineffective in other breast cancer cells which lack an estrogen receptor and (*b*) antiestrogen effects are completely reversed by subsequent addition of estrogens, suggests that the antiestrogen effects on human breast cancer are mediated through interaction with estrogen receptor and are not due to nonspecific and toxic effects to cells. Tamoxifen binds to receptor with approximately 20–100-fold lower apparent affinity than estradiol. Apart from the difference in binding affinity of the ligand for receptor, no significant difference in the initial binding and translocation of cytoplasmic estrogen receptor by antiestrogens has been reported. However, various antiestrogens differ substantially from one another in their dynamics of interaction and in their ability to stimulate increased cellular levels of progesterone receptor. Since most of the antiestrogens tested inhibit MCF-7 cell growth, but differ in their ability to increase cellular progesterone receptor levels, cell growth and progesterone-receptor induction seem to be independently modulated by antiestrogen in these cells (88).

Our group has studied the estrogen antagonistic property of a newly synthesized drug, LY 117018, in MCF-7 cells. This compound has been shown to be more potent than tamoxifen in antagonizing the uterotropic effects of estradiol in rats and mice (90). In MCF-7 cells, the relative binding affinity of LY 117018 appeared to be approximately equal to estradiol, whereas tamoxifen competed about 100 times less well (91). The dissociation constant, as calculated by Scatchard analyses, gave approximately equal values for estradiol and LY 117018. The number of binding sites was also the same. In fact, double reciprocal plots in the same study clearly demonstrated a direct competition between estradiol and antiestrogen for cytoplasmic estrogen receptor and, thus, LY 117018 directly reduced the number of estrogen-receptor complexes translocatable to nuclei and capable of initiating specific estrogenic responses. Moreover, because of the powerful inhibition of all estrogenic functions tested, including complete inhibition of progesterone receptor induction, LY 117018 appears to be a very promising antiestrogen for endocrine therapy in hormone-responsive human breast cancers.

Rochefort et al (87) compared the dissociation rates of estrogen- and antiestrogen-receptor complexes in MCF-7 and other cytosol fractions and suggested a different estrogen-receptor activation by estrogens as compared with antiestrogens. Their study demonstrated that receptor activation through heating at 20°C–25°C resulted in a decreased dissociation rate of estrogen-receptor complexes but not of hydroxytamoxifen-receptor complexes. The consequence of such an impeded activation could be a nonproductive translocation of receptor with defective interaction with chromatin sites. This would explain why the receptor-antiestrogen complex is not fully active and, in some cases, completely unable to induce estrogen-specific proteins. In addition to the difference in the activation mechanism, another major difference in the estrogen and antiestrogen pathways has been reported to occur immediately after translocation to the nucleus: MCF-7 cells incubated with tamoxifen have been reported to process nuclear receptor to a lesser degree than those cells incubated with estradiol, while cells exposed to nafoxidine demonstrated no receptor processing at all. Failure of receptor processing has been suggested to relate to antiestrogenic effects (62–64). In another recent study, Eckert and Katzenellenbogen (92), using two high-affinity radiolabeled antiestrogens, ^{3}H-CI628M and ^{3}H-hydroxytamoxifen, have reported similar physicochemical properties of cytoplasmic estrogen receptor in MCF-7 cytosol (sedimentation coefficient of ∼ 4.1S, Stokes radius of ∼ 4.39S nm, and molecular weight of ∼ 76,000), regardless of the ligand used to label the receptor. Each compound was bound to a single class of high-affinity binding sites, with a Kd of ∼ 0.13 nM for estradiol, ~ 0.14 nM for hydroxytamoxifen, and 0.22 nM for CI628M. However, they found a marked alteration in the molecular properties of nuclear estrogen- and antiestrogen-receptor complexes. The nuclear antiestrogen-receptor complex sedimented at 5.5S and eluted with a Stokes radius of 5.93 nm (molecular weight of 137,000), whereas the nuclear estradiol-receptor complex sedimented at 4.1S and had a Stokes radius of 4.8 nm (molecular weight of 83,000). Furthermore, the shift in sedimentation rate and size of the nuclear receptor was reversible by 3 M urea, yielding estrogen and antiestrogen complexes with molecular weight of 81,000 (3.9S, 4.94-nm Stokes radius). These findings suggest that antiestrogens may promote a molecular association of estrogen receptor with another cellular component with molecular weight of ~ 55,000, resulting in a transformed conformational state of antiestrogen-receptor complex (92). This new complex may be biologically nonproductive and would block the receptor from associating with biologically important chromatin binding or acceptor sites. Additionally, or alternatively, this transition to an altered nuclear form, which may involve physical relocation, could prevent entry into or exit from nuclear processing sites. Thus, taken together, the antiestrogen effect could be exerted by blocking occupation of key chromatin acceptor sites by an inactive antiestrogen-receptor complex or through a depletion of cytoplasmic receptor, preventing further translocation of estrogen-receptor complex into nucleus. Moreover, further studies are needed to understand their effect on estrogen-specific gene and gene products.

Recently, a specific class of high-affinity saturable cytoplasmic binding sites for triphenylethylene antiestrogens, which are distinct from the estrogen-binding site, has been described in normal and neoplastic cells, suggesting that antiestrogens may act via other receptor sites specific for antiestrogen (93,94). In fact, the data show that structural determinants for the binding of nonsteroidal antiestrogens to the estrogen receptor and the specific antiestrogen-binding sites are different in MCF-7 cell cytosol. The antiestrogen-binding protein was observed when the cells were grown in the presence of 10% fetal calf serum, which saturates estrogen-receptor sites. Jozan et al (95) reported a similar finding, noting 12,000 antiestrogen-specific sites/cell in MCF-7 cells, which were saturable and had high affinity. Interestingly, the level of these antiestrogen-specific sites was decreased to 2700 sites/cell in a tamoxifen-resistant variant. On the other hand, the concentration of estrogen receptor remained unchanged, both in the wild and variant cell lines (48,000 and 43,000 sites/cell, respectively), suggesting that antiestrogen-binding sites may have a role in regulating the effect of estrogens.

The effective competitive displacement of estrogen binding with antiestrogens and the phenomenon of estradiol rescue of antiestrogen-treated cells (89), supported by the direct labeling experiments of Eckert and Katzenellenbogen (92), strongly favor that antiestrogens control the growth of breast cancer by competing with estradiol on estrogen receptor, rather than through a separate antiestrogen-binding site. Recently, Coezy et al (96) have addressed the nature of the receptor responsible for antiestrogen and antitumoral activity in MCF-7 cells. Using direct labeling with ^{3}H-tamoxifen and separate competitive binding studies with ^{3}H-estradiol-receptor complex from MCF-7 cytosol, they have clearly shown that the ability of tamoxifen and hydroxytamoxifen to inhibit the MCF-7 cell growth correlates with their binding affinity for estrogen receptor. Therefore, antiestrogens probably control the growth of breast cancer by interacting directly with estrogen receptor, rather than with a separate antiestrogen-binding protein. Furthermore, using both a polyclonal and a monoclonal antibody to calf uterine estrogen-receptor complex, a recent study by Garcia et al (97) gives additional evidence for a direct interaction of antiestrogen with estrogen receptor.

Genetic Analysis of Estrogen and Antiestrogen Action in MCF-7 Variants

Despite the fact that a correlation between the presence of receptor activity and hormone response of breast tumors has been shown, not all breast cancers containing estrogen receptor respond to endocrine therapy. In such tumors, a defect in the estrogen-receptor pathway beyond the initial binding of hormone to receptor is likely. The MCF-7 cell line, which contains estrogen receptors, is estrogen-responsive, and is inhibited by antiestrogens, is ideally suited to study of the molecular mechanism of estrogen and antiestrogen action. In fact, the use of an appropriate tissue culture cell line allows cellular genetic analysis to be applied to supplement biochemical observations. Such genetic studies require that a culture system is available, which permits the selection of mutant or variant cells with altered phenotype. In this direction, development of glucocorticoid-resistant S49 lymphoma cell lines has added a novel dimension in understanding the molecular mechanism of glucocorticoid action in these cells (98,99). Interestingly, virtually all of the steroid-resistant clones have apparently been receptor mutants with multiple defects, including loss of binding activity, thermolabile receptors, defective nuclear translocation, and DNA affinity abnormalities. In fact, in complementation studies, the glucocorticoid responsiveness could not be restored by hybridization between unresponsive cell lines, suggesting that all of the phenotypes were due to defects inherent in the receptor molecule.

We chose a similar approach for MCF-7 estrogen-responsive cells and developed estrogen- and antiestrogen-resistant variants from these hormone-responsive, wild type, parental cells. Two reviews of this subject have recently been published (100,101). In brief, MCF-7 cells were cloned in soft agar in the continuous presence of lethal concentrations of tamoxifen (10^{-6} M). Surprisingly, the majority of the clones were inhibited by tamoxifen, as with the wild type cells. However, two clones exhibited markedly different responses to the inhibitory effects of tamoxifen and demonstrated a variety of altered, but interesting, biologic responses (102). It is important to note that both of these clones had chromosomal patterns and allozyme phenotypes identical to wild type MCF-7 cells. One of these clones, termed R27, retained responsiveness to estrogens (in terms of growth stimulation), but evinced a complete loss of inhibitory antiestrogenic responses. Interestingly, R27 contained unoccupied receptors in both cytoplasm and nuclear extracts, with dissociation constant, sedimentation behavior, and molecular weight characteristics similar to those of MCF-7 receptors. Moreover, R27 contained normal amounts of progesterone receptor, which was inducible by physiologic concentration of estradiol. Also, the extent of competition with estradiol and tamoxifen for the cytoplasmic estrogen receptor was the same for R27 and MCF-7. However, in this cell line, activation of receptor in vitro was different from wild type MCF-7 cells. Under all conditions employed (temperature, salt, and nucleotides), R27 showed a decreased, but not absent, ability to be activated as assessed by the binding of activated estrogen-receptor complex to DNA-cellulose. Moreover, R27 also had a defect in receptor processing. Processing is partially impaired in MCF-7 cells when tamoxifen is added; however, while showing normal nuclear translocation of receptor with tamoxifen, R27 failed to show processing with tamoxifen. Additionally, the effect of estrogen and antiestrogen on the secretion of glycoprotein (52,000 molecular weight) and progesterone receptor was studied in MCF-7 and R27 cells (100). Estrogen stimulated and antiestrogen inhibited the synthesis of these proteins in wild type cells. In R27, these proteins were stimulated by estrogen, but paradoxically, antiestrogen also stimulated the synthesis and secretion of progesterone receptor and the 52,000-dalton protein. This stimulatory effect of antiestrogen in R27 may be analogous to the situation in those patients who have been described as having a stimulation of their breast tumor after antiestrogen treatment. Thus, these characterization studies in R27 suggested that R27 has some alteration in receptor, exhibiting a defect distal to the initial binding of tamoxifen to the cytoplasmic receptor.

The second clone, which was also selected by cloning wild type MCF-7 cells in soft agar in the presence of 10^{-6} M tamoxifen, has been termed R3 (103). This estrogen-independent clone had both cytoplasmic and nuclear receptor binding, with molecular characteristics similar to the estrogen receptor in MCF-7 cells. Moreover, receptor activation and translocation to the nucleus were equivalent in R3 and MCF-7 cells. Relative binding affinity and the extent of competition by estradiol and tamoxifen for cytosolic receptors were the same for R3 and the wild type cells. However, R3 failed to show nuclear-receptor processing and, therefore, had no detectable progesterone receptor inducible by estradiol. In addition to this functional defect, we found that R3 had altered binding to DNA-cellulose versus that of MCF-7 cells; the receptor binding consistently eluted at a higher salt concentration. In mixing experiments, both receptor phenotypes were still apparent. Therefore, R3 is probably another receptor variant with deranged receptor function and is analogous to the clinical situation in which a patient's tumor sample is found to be positive for estrogen receptor, but the patient fails to exhibit a response to endocrine therapy.

Thus, characterization of such resistant cells which are hormonally unresponsive or less responsive can provide discriminatory probes for receptor functionality and thereby make possible identification of hormone-dependent and hormone-independent tumors. Genetic analysis of other variants should provide a more exact knowledge of how estrogens and antiestrogens can specifically alter gene regulation in hormone-dependent tissues and exhibit their diverse phenotypic effects. It will be interesting to study if this approach will allow selection of genetically complementable lesions in other steps of the hormone-receptor pathway, therefore suggesting new routes for biochemical analysis.

More recently, another variant of the MCF-7 cells was selected in our laboratory by exposing cells to high-specific-activity 16α-iodoestradiol. These cells were viably frozen for several isotopic half-lives, defrosted once, then re-exposed to allow maximal radiation damage mediated by the isotope associated with binding sites. This cell line, I-13, was one of 55 clones isolated from MCF-7 that survived this treatment. The growth response of these cells to estradiol was considerably altered. In the presence of 1 nM estradiol, the cells grew normally for 4-6 days, then the cell number plateaued as the cells appeared to round up and detach. Concomitantly, a decrease in ^{3}H-thymidine incorporation occurred. The inhibition was dose-dependent and can be seen in concentrations of estradiol as low as 0.1 nM. The I-13 cells had normal levels of cytosol estrogen receptors, which were capable of nuclear translocation. Progesterone receptor was estrogen-inducible, although the levels were one-third that of wild type MCF-7 cells. Whether the defect in the I-13 cell line is receptor-mediated or related to specific gene products remains to be elucidated.

Acceptor Sites

An unexplained aspect of steroid induction of gene activity has been the direction of hormone-receptor complexes to the desired target genes. One paradigm involves translocated hormone-receptor complex interaction with putative high-affinity acceptor sites on chromatin, located in or near structural genes or regulatory sites. The acceptor sites would confer finer selectivity to the hormone-receptor complex for interaction with specific DNA sequences, whose transcription is to be induced by the hormone. Data on these nuclear binding sites are very controversial and the precise nature of the acceptor sites is not known. Mechanistically, the acceptor may be a protein, RNA, DNA, or a complex of these, but there is a surfeit of evidence that suggests that every component in the nucleus possesses nuclear-receptor binding characteristics in a cell-free system. The relationship between receptor-acceptor interaction and induction of specific response appears to be complex and multifaceted. A growing interest has arisen as to the nature of nuclear binders, which mediate specific gene expression in target tissues. In this direction, Spelsberg and co-workers have long been interested in elucidating the precise nature and function of putative acceptor sites for progesterone-receptor and, more recently, estrogen-receptor complexes of chick oviduct. In one of their earlier studies, Spelsberg et al (104) demonstrated that one group of acid nonhistone proteins, designated as AP_3, appeared to be most effective in conferring chromatin binding ability to chick oviduct progesterone-receptor complex. This AP_3 fraction, when selectively removed from or reconstituted with AP_3-deleted chromatin, caused a loss or restoration of acceptor activity. In a series of interesting experiments, it was shown that progesterone-receptor binding specificity can be conserved in reconstituted chromatin only if target tissue nonhistone chromosomal proteins are used; the nature of DNA and histones made no contributions to this selectivity (105). All such studies imply that DNA and tissue specificity reside in these acidic proteins. Further fractionation studies revealed acceptor activity to be associated with two hydrophobic proteins with molecular weights between 12,000 and 17,000. These tissue-specific acceptor proteins, upon reannealing to DNA, act as coacceptors for high-affinity binding to progesterone receptor. Recently, Spelsberg (106) has extracted this residual nonhistone protein-DNA complex called nucleoacidic protein (NAP) by the extraction of chromatin with 4 M guanidine hydrochloride. Binding to NAP was receptor-dependent saturable, specific, and exceeded binding to pure DNA, suggesting that NAP may contain putative acceptor sites involved in the selective binding of progesterone receptor to the avian genome.

Similarly, in the estrogen-receptor system, studies aimed at explaining the chromosomal association and distribution of estrogen-receptor complex in MCF-7 cells were reported by Scott and Frankel (107). With micrococcal nuclease digestion of MCF-7 cell nuclei, preferential binding of estrogen receptor in transcriptionally active chromatin was shown. This fraction was enriched in mononucleosomes, contained nonhistone chromosomal proteins, and was depleted in histone H_1. Thus, association of receptor with chromatin enriched in transcribed sequences implied preferential, not random, distribution of estrogen receptor to MCF-7 chromatin. Besides involvement of nonhistone protein, Kallos and co-workers (108) have recently shown that structural chromosomal histones, around which genes are packaged, are also implicated in the molecular mechanism of steroid-induced gene activation. Their study has demonstrated that estrogen receptor from rabbit uterus binds with high affinity to histones H_2A and H_2B, moderately to H_3 and H_4, but poorly to histone H_1. Most experiments of others, however, suggest that histones may not be the specific acceptor molecules involved in selective interaction with receptors. Whether the receptor first recognizes the acceptor and then the acceptor steers the complex to unique-sequence genes or whether the receptor directly binds to DNA and the acceptor directs the specificity is still unknown. Finally, each target cell appears to contain thousands of nuclear acceptor sites; the significance of this acceptor redundancy has not been addressed. Thrall et al (105) have shown a class of nuclear proteins, called repressing protein fraction, that normally mask most of the chromatin acceptor sites, suggesting a possible regulation of acceptor activity at the chromosomal level. It is attractive to consider that redundancy of acceptor sites may provide a dual regulatory process, one in the acceptor activity (positive regulation) and a second in the repression of activity (negative regulation).

When perfected, such an acceptor assay will undoubtedly have a significant role in selective use of endocrine therapy for patients with breast cancer. Identification of such a selective nuclear interaction, which is distal to the hormone binding with the cytosolic receptor, would be an invaluable aid in predicting the hormone-responsiveness of breast tumors, particularly those which are receptor-positive but fail to respond to endocrine therapy. It is possible that such receptor-positive transformed cells may have masked genomic acceptor sites, thus resulting in impeded receptor-acceptor interaction. It will be equally informative to compare the acceptor sites for estrogen receptor in hormone-dependent and autonomous tumors to understand hormone-regulated gene expression. Further, since receptor translocation to nucleus is normal for a number of antiestrogens, the chromatin acceptor assay should be discriminatory and help in advancing the application of antiestrogens to newer treatment approaches for human breast cancer.

Steroid Receptors and Modulation of Gene Expression

During the past 5 years, there has been a remarkable advancement in the contributions made towards defining the molecular mechanism of steroid hormone action. In this section, we review studies that seek to characterize the nature of receptor-DNA interaction and to generate a possible mechanism to define hormone-mediated regulation of eukaryotic gene expression.

Following translocation of the hormone-receptor complex to the nucleus, one central question that remains to be answered concerns the nature of the nuclear sites with which the hormone-receptor complex eventually interacts. Whatever they may be, it is tempting to suggest that binding of the complex to these sites is tightly coupled with the apparent changes in gene expression. Some earlier studies by Gorski and Gannon (1) suggested the existence of multiple sites of receptor interaction. This is supported by the fact that steroid hormone receptors bind to DNA (109), RNA (110), chromatin (106), nuclear matrix (111), and nuclear envelope (112). Additionally, several studies have shown that the nuclear binding is nonsaturable and is a linear function of receptor-steroid complex concentration (113). If the bulk of the receptors associate nonspecifically with nuclear components, then the immediate question that needs to be addressed is how a nonspecific low-affinity type of interaction could eventually result in the regulation of a small number of specific genes that are hormonally induced. To this end, Yamamoto and Alberts (3) proposed that besides an association with low-affinity nonspecific sites, an additional interaction with a small number of high-affinity sites leads to productive gene activation and directly mediates the selectivity of hormone action. In fact, the large excess of low-affinity sites could mask the detection of the small number of high-affinity genomic binding sites. This could explain why, in most instances, the binding of steroid-receptor complexes to DNA was reported to be nonsaturable. Since in all the previous studies total DNA has been used, the lack of DNA specificity could be due to the inability to detect a few high-affinity sites among an overwhelming number of low-affinity DNA sites. The net effect of hormone action would be to alter the pattern of cellular proteins synthesized, probably by altering the transcription of a few specific genes. Furthermore, it is now well-documented that the steroid-receptor complex has DNA-binding characteristics which may be tightly related to eliciting a biologic response (2,3). However, to date, there is no direct evidence showing that such interactions lead to changes in transcription or that steroid-receptor complexes must associate with particular DNA sequences to modulate gene expression.

The regulation of mouse mammary tumor virus (MMTV) expression provides an excellent experimental system for the study of steroid hormone action. In both mouse mammary tumor cells and infected heterologous cells, the level of MMTV RNA and virus can be stimulated by dexamethasone acting through a cytoplasmic receptor protein (114,115). The stimulation of synthesis of viral RNA, which is rapid and independent of apparent protein synthesis, is due to an increase in the rate of primary transcription of MMTV proviral DNA, suggesting that dexamethasone exerts its effect on MMTV directly at the viral RNA transcription level (116).

The recent availability of cloned fragments of hormonally induced genes and excellent advances in recombinant DNA technology and somatic cell genetics have vastly added to a more detailed understanding of the molecular mechanism of hormonal regulation of eukaryotic transcription. Several groups have reported on the direct analysis of the selective interaction of hormone-receptor complexes with DNA. Using nitrocellulose membrane assays, Payvar et al (117) have shown that partially purified activated glucocorticoid receptor binds selectively in vitro with some, but not all, portions of a cloned fragment of MMTV DNA. MMTV DNA, like other retroviruses, integrates at many sites of the genome of receptor-containing cells, which implies that the hormonal regulation of MMTV gene expression is not site-specific. Recently, several groups have shown independently that the long terminal repeats (LTR) of MMTV contain a region which confers glucocorticoid responsiveness. Using filter-binding assay and visualization of the complexes by electron microscopy, Govindan et al (118) have shown specific binding of purified glucocorticoid receptor hormone complex to the LTR of the cloned MMTV DNA. More recently, using a DNA-cellulose competition assay, Pfahl (119) has tried to localize the hormone-receptor binding sequence within the MMTV LTR. In this study, a 550 bp pst I fragment of the 5′ LTR of the proviral DNA showed five- to six-fold higher affinity for rat and mouse glucocorticoid receptors. This 550 bp fragment contains promoter signals and a transcription start site. A similar observation on receptor-DNA interaction has been reported by Mulvihill and co-workers (120). Using a calf-thymus DNA-cellulose competition assay, a preferential binding of chicken oviduct progesterone-receptor complex to cloned 5′- terminal ovalbumin gene has been detected. Perhaps the most interesting finding from the direct DNA-binding data of Mulvihill and co-workers has been the identification of a 48 bp stretch, located 250-300 bp upstream from the mRNA start site of the ovalbumin gene, which most efficiently displaces progesterone-receptor complex bound to calf-thymus DNA-cellulose. Furthermore, sequence homologies with this 48 bp region were observed in other fragments of hormonally regulated genes (conalbumin, ovomucoid, x and y), which also compete effectively. The comparison of sequences in this 48 bp region with the DNA sequences of other cloned fragments showing positive competition permitted them to construct a consensus sequence (ATC $\genfrac{}{}{0pt}{}{\mathrm{CC}}{\mathrm{TT}}$ ATT $\genfrac{}{}{0pt}{}{\mathrm{A}}{\mathrm{T}}$ TCTG $\genfrac{}{}{0pt}{}{\mathrm{G}}{\mathrm{T}}$ TTGTA) of 19 bp, identified at the most probable receptor-binding site. Concurrently, using about 10,000-fold purified hen oviduct progesterone receptor A, studies by Compton et al (121) have reported about a tenfold binding preference for DNA fragments flanking the 5′ end of ovalbumin gene. Detailed mapping of the receptor-binding site in this 5′ flanking region revealed a preferential binding sequence, which is 114 bp long and lying between -247 and -135 bp upstream from the start of ovalbumin gene transcription. Perhaps the most interesting characteristic of this 114 bp stretch is the presence of highly rich A + T 18 bp region, which may be a likely genomic site for progesterone-receptor interaction.

Mechanistically, it appears that all the steroid receptors interact specifically near the 5′ end of the cellular genes to modulate the expression in a common manner. However, such studies on protein-DNA interaction need to be more rigorously tested. In this type of direct study, the availability of a homogeneous receptor preparation is important, since filter paper binding may result from any adhering contaminant that may co-purify with receptor and selectively associate with a hormone-regulated DNA sequence. Aside from the difficulty in purifying steroid receptor, it is worth considering that purification may be accompanied by alterations in receptor conformation or loss of a cofactor, which may change the sequence preference. Finally and most importantly, in the absence of obligatory chromosomal proteins, as discussed in the preceding section, the selective binding to naked linear DNA may differ substantially from binding to the DNA in chromatin in situ. Unless some direct visualization by electron microscopy is made to identify the sites involved in vivo which result in a transcriptionally productive conformation, it is difficult to predict how best they reflect the in vivo situation. The reason for multiplicity of receptor interaction in the same genetic region has not yet been addressed; clearly, it will be important to test whether productive gene activation requires a large contiguous stretch of altered chromatin structure, which is achieved only from multiple binding, or whether the interaction is with a single monospecific highly enriched site which may be the reason for selectivity of biologic response.

Even after the binding of receptor molecule(s) directly to a specific patch of nucleotide sequence it potentially regulates, the question that remains is how this genomic occupancy is temporally coupled to the induction of specific gene product. One possible mechanism may be that this specific binding is analogous to the binding of lac and lambda repressors to gene operator regions or *Escherichia coli* gene activator protein and *E. coli* RNA polymerase to promotor sites. Similar to receptor-DNA interactions, prokaryotic regulatory proteins, including *E. coli* RNA polymerase, bind with moderate affinity to nonspecific DNA sequences, in addition to their tight binding to specific control regions within DNA. When the entire genome is tested, selective binding is undetectable but becomes apparent only when the binding region is selectively enriched relative to nonspecific sites. Similarly, binding of SV-40 T-antigen to specific sequence on SV-40 DNA may provide an analogous transcriptional regulatory mechanism. This type of selective binding may make the promoter more efficient or may facilitate the polymerase-induced melting transition of adjacent sequence required for start of RNA synthesis. It is also possible that DNA sequences may not be close to the steroid-regulated structural genes, but could transcribe products which may subsequently regulate the structural gene through a cascade mechanism. It will be interesting to determine if the receptor acts catalytically or stoichiometrically to induce specific transcription. This information will answer the important question of whether the constant presence of the receptor is necessary for the gene to be stimulated. Since chromatin structure, which confers nuclease sensitivity to expressible genes, contains both transcribed and nontranscribed spacer elements, it is possible that the receptor-genomic interaction changes the contiguous patch of chromatin to influence gene expression. Furthermore, not only because of preferential receptor binding to single-stranded DNA or to nicked DNA, but also because the receptor binding increases susceptibility of double-stranded DNA to nuclease S1 digestion, it is possible that the receptor may function as a DNA-helix destabilizing protein at a proximal A + T rich region (121,122). This DNA-helix melting may, in turn, be involved with replication, recombination, repair, and transcription. In fact, the purified subunit A of chick oviduct progesterone receptor shows several properties of a DNA-helix destabilizing protein (122).

Another possible mechanism by which hormones could influence specific gene expression is by inducing demethylation at the control region of the hormone-inducible genes. Numerous reports indicate that 2%-7% of the cytosine residues, generally in a CG sequence within DNA, are enzymatically converted to 5-methylcytosine shortly after DNA replication by methyl transfer from S-adenosylmethionine to position 5 of cytosine. This DNA methylation has been proposed to be involved in specific gene regulation and differentiation. Reports suggesting its involvement in other possible functions, including repair of DNA, recombination, and mutation (in *E. coli*, methylated sites can be mutation "hot spots") and changing the chromosome structure (by permitting a transition from Z to the B configuration of DNA) are forthcoming. Studies of the last 3-4 years, using methylation-sensitive restriction enzymes, suggest an inverse correlation between the extent of DNA methylation and the expression of specific genes, although there are some exceptions. In many cellular genes, including globin, ovalbumin, conalbumin, ovomucoid, and metallothionein, it has been found that the expressed genes are undermethylated and the repressed genes are more extensively methylated. Recent studies by Wilks and co-workers (123) demonstrate that in the oviduct and liver of egg-laying hens, the vitellogenin gene becomes demethylated at its 5′ end after estrogen stimulation, even though the oviduct does not synthesize vitellogenin mRNA or precursor mRNA. Therefore, in this system, estrogen induces demethylation of the vitellogenin gene, regardless of whether or not the gene is expressed. Another recent study describes the glucocorticoid-regulated expression of MMTV and demethylation of LTR repeat sequences of MMTV in a T-lymphoid cell line sensitive to glucocorticoid-induced lymphocytosis and in two of its variants, one that is supersensitive and the other that is resistant to glucocorticoid-killing effect (124). In both the parental cell line and the supersensitive variant (both being receptor-positive), MMTV expression is induced after short treatment with dexamethasone. However, the induction of MMTV mRNA is reduced in the resistant line, which is also defective in glucocorticoid receptors. The striking observation of this study is that the resistant variant showed a very high basal level of MMTV mRNA in the absence of dexamethasone. The interpretation of this observation is that, since the resistant variant was selected by prolonged treatment with high concentrations of dexamethasone, the continuous transcription of MMTV during this selection period could have resulted in the demethylation at the LTR sequences. Therefore, this variant has a high constitutive expression of MMTV in the absence of the hormone. Demethylation was not detected after short exposure of the parental cell line to dexamethasone, suggesting that demethylation may occur as a result of continuous hormone treatment. On the other hand, in the case of chicken erythrocytes, although the globin gene is not continuously transcribed, it is still undermethylated and also sensitive to DNase I. Therefore, it is not clear whether DNA demethylation should precede or follow active gene expression.

In summary, using DNA recombinant technology, this type of direct approach could be wisely exploited for cloning the hormone-responsive genes in human mammary carcinoma cells that display varying response to estrogen, androgen, glucocorticoid, and progestins. In this direction, the MCF-7 cell line (which is not only stimulated to divide by estrogens, but is growth-inhibited by glucocorticoids and antiestrogens) provides a particularly exciting model for studying gene regulation in hormone-responsive human breast cancers. Furthermore, it may be possible to map in greater detail the putative regulatory genomic elements by reintroducing cloned DNA, either bearing or lacking the receptor-binding sites, into receptor-containing and receptor-defective cells, to assess their competence for hormone responsiveness.

REFERENCES

1. GORSKI J, and GANNON F. Current models of steroid hormone action: a critique. Annu Rev Physiol 38:425-450, 1976.
2. O'MALLEY BW, SCHWARTZ RJ, and SCHRADER WT. A review of regulation of gene expression by steroid hormone receptors. J Steroid Biochem 7:1151-1159, 1976.
3. YAMAMOTO KR, and ALBERTS BM. Steroid receptors: elements for modulation of eukaryotic transcription. Annu Rev Biochem 45:721-746, 1976.
4. KATZENELLENBOGEN B. Dynamics of steroid hormone receptor action. Annu Rev Physiol 42:17-35, 1980.
5. SCHRADER WT, BIRNBAUMER ME, HUGHES MR, ET AL. Studies on the structure and function of the chicken progesterone receptor. Recent Prog Horm Res 37:583-633, 1981.
6. JENSEN EV, and JACOBSON HI. Basic guides to the mechanism of estrogen action. Recent Prog Horm Res 12:387-414, 1962.
7. NOTEBOOM WD, and GORSKI J. Stereo-specific binding of estrogens in the rat uterus. Arch Biochem Biophys 111:559-568, 1965.
8. TOFT D, and GORSKI J. A receptor molecule for estrogen: isolation from the rat uterus and preliminary characterizations. Proc Natl Acad Sci USA 55:1574-1581, 1966.
9. SHERMAN MR, CORVOL PL, and O'MALLEY BW. Progesterone-binding components of chick oyiduct: 1. Preliminary characterization of cytoplasmic components. J Biol Chem 245:6085-6096, 1970.
10. BAXTER JD, ROUSSEAU CG, BENSON MC, ET AL. Role of DNA and specific cytoplasmic receptors in glucocorticoid action. Proc Natl Acad Sci USA 69:1892-1896, 1972.
11. THOMPSON EB, VENETIANER A, GELEHRTER TD, ET AL. Multiple actions of glucocorticoid studies in cell culture systems. *In* Gene Regulation by Steroid Hormones (Roy A, and Clark J, eds). New York, Springer-Verlag, 1980, pp 122-152.
12. BRUMBAUGH PF, HUGHES MR, and HAUSSLER MR. Cytoplasmic and nuclear binding components for 1α,25-dihydroxyvitamin D_3 in chick parathyroid glands. Proc Natl Acad Sci USA 72:4871-4875, 1975.
13. EISMAN JA, MARTIN TJ, MACINTYRE RJ, ET AL. 1,25 Dihydroxy vitamin D_3 receptor in a cultured human breast cancer cell line (MCF-7 cells). Biochem Biophys Res Commun 93:9-15, 1980.
14. EDELMAN IS, and FIMOGNARI GD. On the biochemical mechanism of action of aldosterone. Recent Prog Horm Res 24:1-44, 1968.
15. YOUNG MA, KING DA, and FRISTROM JW. Ecdysteroid receptors in imaginal discs of *Drosophila melanogaster*. Proc Natl Acad Sci USA 75:6039-6043, 1978.
16. GRODY WW, SCHRADER WT, and O'MALLEY BW. Activation, transformation, and subunit structure of steroid hormone receptors. Endocr Rev 3:141-162, 1982.
17. YAMAMOTO KR. Characterization of the 4S to 5S forms of the estradiol receptor protein and their interaction with deoxyribonucleic acid. J Biol Chem 249:7068-7075, 1974.
18. SIITERI PK, SCHWARZ BE, MORIYAMA I, ET AL. Estrogen binding in the rat and human. Adv Exp Med Biol 36:97-112, 1973.
19. ENGEL LW, and YOUNG NA. Human breast carcinoma cells in continuous culture: a review. Cancer Res 38:4327-4339, 1978.
20. SOULE HD, VAZQUEZ J, LUNG A, ET AL. A human cell line from a pleural effusion derived from a breast carcinoma. JNCI 51:1409-1416, 1973.
21. KNAZEK R, LIPPMAN ME, and CHOPRA H. Formation of solid human mammary carcinoma in vitro. JNCI 58:419-428, 1977.
22. SEIBERT K, SHAFIE SM, TRICHE TJ, ET AL. Clonal variation of MCF-7 breast cancer cells in vitro and in athymic nude mice. Cancer Res, in press.
23. ROSE HN, and MCGRATH CM. Lactalbumin production in human mammary carcinoma. Science 190:673-674, 1975.
24. CAILLEAU R, YOUNG R, OLIVE M, ET AL. Breast tumor cell lines from pleural effusions. JNCI 53:661-674, 1974.
25. HORWITZ KB, COSTLOW ME, and MCGUIRE WL. MCF-7: a human breast cancer cell line with estrogen, androgen, progesterone and glucocorticoid receptors. Steroids 26:785-795, 1975.
26. HORWITZ KB, ZAVA DT, THILAGER AK, ET AL. Steroid receptor analyses of nine human breast cancer cell lines. Cancer Res 38:2434-2437, 1978.
27. LIPPMAN M, MONACO ME, and BOLEN G. Effects of estrone, estradiol, and estriol on human-responsive human breast cancer in long-term tissue culture. Cancer Res 37:1901-1907, 1977.
28. LIPPMAN ME, and BOLAN G. Oestrogen-responsive human breast cancer in long-term tissue culture. Nature 256:592-593, 1975.
29. KEYDAR I, CHEN L, KARBY S, ET AL. Establishment and characterization of a cell line of human breast carcinoma origin. Eur J Cancer 15:659-670, 1979.
30. HORWITZ KB, MOCKUS MB, and LASSEY BA. Variant T47D human

breast cancer cells with high progesterone-receptor levels despite estrogen and antiestrogen resistance. Cell 28:633-642, 1982.

31. MOCKUS MB, LESSEY BA, BOWES MA, ET AL. Estrogen-insensitive progesterone receptors in a human breast cancer cell line: characterization of receptors and of a ligand exchange assay. Endocrinology 110:1564-1571, 1982.
32. YU W, LEUNG BS, and GAO YL. Effects of 17β-estradiol on progesterone receptors and the uptake of thymidine in human breast cancer cell line CAMA-1. Cancer Res 41:5004-5009, 1981.
33. SOULE HD, VAZQUEZ J, LUNG A, ET AL. A human cell line from a pleural effusion derived from a breast carcinoma. JNCI 51: 1409-1416, 1973.
34. RUSSO J, SOULE HD, MCGRATH C, ET AL. Reexpression of the original tumor pattern by a human breast carcinoma cell line (MCF-7) in sponge culture. JNCI 56:279-285, 1976.
35. KNAZEK R, LIPPMAN ME, and CHOPRA H. Formation of solid human mammary carcinoma in vitro. JNCI 58:419-428, 1977.
36. SEIBERT K, SHAFIE SM, TRICHE TJ, ET AL. Clonal variation of MCF-7 breast cancer cells in vitro and in athymic nude mice. Cancer Res, in press.
37. BRONZERT DA, MONACO ME, PINKUS L, ET AL. Purification and properties of estrogen responsive cytoplasmic thymidine kinase from human breast cancer. Cancer Res 41:604-610, 1981.
38. LACROIX A, and LIPPMAN M. Binding of retinoids to human breast cancer cell line and their effects on cell growth. J Clin Invest 65:586-591, 1980.
39. OSBORNE CK, HAMILTON B, TITUS G, ET AL. Epidermal growth factor stimulation of human breast cancer cells in culture. Cancer Res 40:2631-2636, 1980.
40. BARNES D, and SATO G. Growth of a human breast cancer cell line in a serum-free medium. Nature 281:388-389, 1979.
41. ALLEGRA JC, and LIPPMAN ME. Growth of a human breast cancer cell line in serum-free hormone supplemented medium. Cancer Res 38:3823-3829, 1978.
42. EISMAN JA, MARTIN TJ, MACINTYRE RJ, ET AL. 1,25 Dihydroxy vitamin D_3 receptor in a cultured human breast cancer cell line (MCF-7 cells). Biochem Biophys Res Commun 93:9-15, 1980.
43. TOFT DO, and O'MALLEY BW. Progesterone binding protein of chick oviduct. V. Interaction of purified progesterone-receptor components with nuclear constituents. J Biol Chem 247:2401-2407, 1972.
44. SCHRADER WT, COTY WA, SMITH RG, ET AL. Purification and properties of progesterone receptors from chick oviduct. Ann NY Acad Sci 286:64-80, 1977.
45. MILLER JB, and TOFT DO. Requirement for activation in the binding of progesterone receptor to ATP-Sepharose. Biochemistry 17:173-177, 1978.
46. NOTIDES AC. Conformational forms of the estrogen receptor. *In* Receptors and Hormone Action (O'Malley BW, ed). New York, Academic Press, 1978, pp 33-61.
47. SATO B, NISHIZAWA Y, NOMA K, ET AL. Estrogen-independent nuclear binding of receptor protein of rat uterine cytosol by removal of low molecular weight inhibitor. Endocrinology pp 1474-1479, 1979.
48. BAILLY A, SALLAS N, and MILGROM E. A low molecular weight inhibitor of steroid receptor activation. J Biol Chem 252:858-863, 1977.
49. SIMONS SS, JR, MARTINEZ HM, GARCEA RL, ET AL. Interactions of glucocorticoid receptor activation. J Biol Chem 251:334-343, 1976.
50. PUCA GA, NOLA E, SICA V, ET AL. Estrogen binding proteins of calf uterus: molecular and functional characterization of the receptor transforming factor: a Ca^{2+}-activated protease. J Biol Chem 252:1358-1366, 1977.
51. ANDRE J, RAYNAUD A, and ROCHEFORT H. Characterization of the estradiol receptor extracted from nuclei by micrococcal nuclease. Biochemistry 17:3619-3626, 1978.
52. LEACH KL, DAHMER MK, HAMMOND ND, ET AL. Molybdate inhibition of glucocorticoid receptor inactivation and transformation. J Biol Chem 254:11884-11890, 1979.
53. SEKULA BC, SCHMIDT TJ, and LITWACK G. Redefinition of modulator as an inhibitor of glucocorticoid receptor activation. J Steroid Biochem 14:161-166, 1981.
54. NISHIGORI H, and TOFT D. Inhibition of progesterone receptor activation by sodium molybdate. Biochemistry 19:77-83, 1980.
55. SHYAMALA G, and LEONARD L. Inhibition of uterine estrogen receptor transformation by sodium molybdate. J Biol Chem 255: 6028-6031, 1980.
56. CHONG M, and LIPPMAN ME. Effect of temperature, nucleotide, and sodium molybdate on activation and DNA binding of estrogen, glucocorticoid, progesterone and androgen receptors in MCF-7 human breast cancer cells. J Recept Res 2:575-600, 1981-1982.
57. ______. Effects of RNA and ribonuclease on the binding of estrogen and glucocorticoid receptors from MCF-7 cells to DNA-cellulose. J Biol Chem 257:2996-3002, 1982.
58. JOHN JK, and MOUDGIL VK. Activation of glucocorticoid receptor by ATP. Biochem Biophys Res Commun 90:1242-1248, 1979.
59. MOUDGIL VK, NISHIGORI H, EESSALU TE, ET AL. Analysis of avian progesterone receptor with inhibitors. *In* Gene Regulation by Steroid Hormones (Roy A, and Clark J, eds). New York, Springer-Verlag, 1980, pp 106-119.
60. LIAO S, SMYTHE S, TYMOCZKO JL, ET AL. RNA-dependent release of androgen and other steroid receptor complexes from DNA. J Biol Chem 255:5545-5551, 1980.
61. HORWITZ KB, and MCGUIRE WL. Estrogen control of progesterone receptor in human breast cancer. J Biol Chem 253:2223-2228, 1978.
62. ______. Nuclear mechanisms of estrogen action: effects of estradiol and antiestrogens on estrogen receptors and nuclear receptor processing. J Biol Chem 253:8185-8191, 1978.
63. ______. Actinomycin D prevents nuclear processing of estrogen receptor. J Biol Chem 253:6319-6322, 1978.
64. ______. Nuclear estrogen receptors: effect of inhibitors on processing and steady state levels. J Biol Chem 255:9699-9705, 1980.
65. CIDLOWSKI JA, and MICHAELIS GA. Alterations in glucocorticoid binding site number during the cell cycle in HeLa cells. Nature 266:643-645, 1977.
66. DEFESI CR, FELS EC, and SURKS MI. Nuclear 3,5,3′-triiodothyronine receptor concentration increases during deoxyribonucleic acid synthesis in partially synchronized BC cell cultures. Endocrinology 111:1156-1163, 1982.
67. REINHARDT CG, and KRUGH TR. A comparative study of ethidium bromide complexes with dinucleotides and DNA: direct evidence for intercalation and nucleic acid sequence preference. Biochemistry 17:4845-4854, 1978.
68. CLARK JH, MARKAVERICH B, UPCHURCH S, ET AL. Nuclear binding of the estrogen receptor: heterogeneity of sites and uterotropic response. Adv Exp Med Biol 117:17-46, 1978.
69. AURICCHIO F, MIGLIACCIO A, and ROTONDI A. Inactivation of oestrogen receptor in vitro by nuclear dephosphorylation. Biochem J 194:569-574, 1981.
70. AURICCHIO F, MIGLIACCIO A, and CASTORIA G. Dephosphorylation of oestradiol nuclear receptor in vitro. A hypothesis on the mechanism of action of non-steroidal anti-oestrogens. Biochem J 198: 699-702, 1981.
71. AURICCHIO F, MIGLIACCIO A, CASTORIA G, ET AL. Biochem Biophys Res Commun 106:149-157, 1982.
72. NIELSEN CJ, SANDO JJ, and PRATT WB. Evidence that dephosphorylation inactivates glucocorticoid receptors. Proc Natl Acad Sci USA 74:1398-1402, 1977.
73. BARNETT CA, SCHMIDT TJ, and LITWACK G. Effect of intestinal alkaline phosphatase, phosphatase inhibitors, and phosphorylated compounds on the rate of activation of glucocorticoid-receptor complexes. Biochemistry 19:5446-5455, 1980.
74. LIPPMAN ME, and THOMPSON EB. Pitfalls in the interpretation of steroid receptor assays in clinical medicine. *In* Steroid Receptors and the Management of Cancer (Thompson EB, Lippman ME, eds). Boca Raton, Florida, CRC Press, 1979, vol 1, pp 235-238.
75. CHAMNESS GC, and MCGUIRE WL. Methods for analyzing steroid receptors in human breast cancer. *In* Breast Cancer 3. Advances in

Research and Treatment (McGuire WL, ed). New York, Plenum Press, 1979, pp 149-197.

76. SCHOLL SM, and LIPPMAN ME. Methods and clinical use of receptor assays. *In* Recent Advances in Breast Cancer (Bonadonna G, ed). In press.
77. GREENE GL, CLOSS LE, FLEMING H, ET AL. Antibodies to estrogen receptor: immunochemical similarity of estrophilin from various mammalian species. Proc Natl Acad Sci USA 74:3681-3685, 1977.
78. GREENE GL, CLOSS LE, DESOMBRE ER, ET AL. Antibodies to estrophilin: comparison between rabbit and goat antisera. J Steroid Biochem 11:333-341, 1979.
79. SHERMAN MR, PICKERING LA, ROLLWAGEN FM, ET AL. Mero-receptors: proteolytic fragments of receptors containing the steroid-binding site. Fed Proc 37:167-173, 1978.
80. COFFER AI, and KING RJB. Antibodies to estradiol receptor from human myometrium. J Steroid Biochem 14:1229-1235, 1981.
81. LOGEAT F, HAI MTV, and MILGROM E. Antibodies to rabbit progesterone receptor: crossreaction with human receptor. 78:1426-1430, 1981.
82. GREENE GL, FITCH FW, and JENSEN EV. Monoclonal antibodies to estrophilin: probes for the study of estrogen receptors. Proc Natl Acad Sci USA 77:157-161, 1980.
83. GREENE GL, NOLAN C, ENGLER JP, ET AL. Monoclonal antibodies to human estrogen receptor. Proc Natl Acad Sci USA 77:5115-5119, 1980.
84. GREENE GL, and JENSEN EV. Monoclonal antibodies as probes for estrogen receptor detection and characterization. J Steroid Biochem 16:353-359, 1982.
85. WRIGHT LJ, FEINSTEIN A, HEAP RB, ET AL. Progesterone monoclonal antibody blocks pregnancy in mice. Nature 295:415-417, 1982.
86. WESTLEY B, and ROCHEFORT H. A secreted glycoprotein induced by estrogen in human breast cancer cell lines. Cell 20:353-362, 1980.
87. ROCHEFORT H, COEZY E, JOLY E, ET AL. Hormonal control of breast cancer in cell culture. *In* Hormones and Cancer (Iaccobelli S, et al, eds). New York, Raven Press, 1980, pp 21-29.
88. KATZENELLENBOGEN BS. Basic mechanisms of antiestrogen action. *In* Hormones, Receptors, and Breast Cancer (McGuire WL, ed). New York, Raven Press, 1978, pp 135-157.
89. LIPPMAN ME, BOLAN G, and HUFF K. Interactions of antiestrogens with human breast cancer in long term tissue culture. Cancer Treat Res 60:1421-1430, 1976.
90. BLACK LJ, and GOODE RL. Uterine bioassay of tamoxifen, trioxifene and a new estrogen antagonist (LY117018) in rats and mice. Life Sci 26:1453-1458, 1980.
91. SCHOLL SM, HUFF KK, and LIPPMAN ME. Antiestrogenic effects of LY 117018 in MCF-7 cells. Endocrinology, in press.
92. ECKERT RL, and KATZENELLENBOGEN BS. Physical properties of estrogen receptor complexes in MCF-7 human breast cancer cells. J Biol Chem 257:8840-8846, 1982.
93. SUTHERLAND RL, MURPHY LC, FOO MS, ET AL. High affinity antioestrogen binding site distinct from the oestrogen receptor. Nature 288:273-275, 1980.
94. MURPHY LC, and SUTHERLAND RL. Modifications in the aminoether side chain of clomiphene influence affinity for a specific antiestrogen binding site in MCF-7 cytosol. Biochem Biophys Res Commun 100:1353-1360, 1981.
95. JOZAN S, ELALAMY H, and BAYARD F. Etude du mecanisme d'action d'un antioestrogene du groupe triphenylethylene sur la croissance de la lignee cellulaire de cancer du sein humain MCF-7 en culture. C R Seances Acad Sci 292:767-770, 1981.
96. COEZY E, BORGNA J-L, and ROCHEFORT H. Tamoxifen and metabolites in MCF-7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. Cancer Res 42:317-323, 1982.
97. GARCIA M, GREENE G, ROCHEFORT H, ET AL. Effect of antibodies to estrogen receptor on the binding of ^{3}H-labeled antiestrogens and androstanediol in the uterus. Endocrinology 110:1355-1361, 1982.
98. BOURGEOIS S, and NEWBY R. Diploid and haploid states of the glucocorticoid receptor gene of mouse lymphoid cell lines. Cell 11:423-432, 1977.
99. YAMAMOTO KR, GEHRING U, STAMPFER MR, ET AL. Genetic approaches to steroid hormone action. Recent Prog Horm Res 32:3-32, 1976.
100. LIPPMAN ME, NAWATA H, BRONZERT D, ET AL. Antiestrogen-resistant variants of hormone-responsive MCF-7 human breast cancer cells. Cold Spring Harbor Conference on Cell Proliferation 9:831-843, 1982.
101. LIPPMAN ME, and NAWATA H. A genetic analysis of estrogen action in human breast cancer cell. *In* The Role of Tamoxifen in Breast Cancer (Iacobelli S, Lippman ME, Robustelli DC, eds). New York, Raven Press, 1982, pp 9-16.
102. NAWATA H, BRONZERT D, and LIPPMAN M. Isolation and characterization of a tamoxifen-resistant cell line derived from MCF-7 human breast cancer cells. J Biol Chem 256:5016-5021, 1981.
103. NAWATA H, CHONG MT, BRONZERT D, ET AL. Estradiol-independent growth of a subline of MCF-7 human breast cancer cells in culture. J Biol Chem 256:6895-6902, 1981.
104. SPELSBERG TC, STEGGLES AW, and O'MALLEY BW. Progesterone-binding component of chick oviduct: chromatin acceptor sites. J Biol Chem 246:4188-4197, 1971.
105. THRALL CL, WEBSTER RA, and SPELSBERG TC. Steroid interaction with chromatin. *In* The Cell Nucleus (Busch H, ed). New York, Academic Press 6:461-529, 1978.
106. SPELSBERG TC. Chemical characterization of nuclear acceptors for the avian progesterone receptor. Biochem Act Horm 9:142-197, 1982.
107. SCOTT RW, and FRANKEL FR. Enrichment of estradiol-receptor complexes in a transcriptionally active fraction of chromatin from MCF-7 cells. Proc Natl Acad Sci USA 77:1291-1295, 1980.
108. KALLOS J, FASY TM, and HOLLANDER VP. Assessment of estrogen receptor-histone interactions. Proc Natl Acad Sci USA 78: 2874-2878, 1981.
109. TOFT D. The interaction of uterine estrogen receptors with DNA. Adv Exp Med Biol 36:85-96, 1973.
110. LIANG T, and LIAO S. Association of the uterine 17β-estradiol-receptor complex with ribonucleoprotein in vitro and in vivo. J Biol Chem 249:4671-4678, 1974.
111. BARRACK ER, HAWKINS EF, ALLEN SC, ET AL. Concepts related to salt resistant estradiol receptors in rat uterine nuclei: nuclear matrix. Biochem Biophys Res Commun 79:829-836, 1979.
112. JACKSON V, and CHALKLEY GR. The binding of estradiol-17β to the bovine endometrial nuclear membrane. J Biol Chem 249:1615-1626, 1974.
113. CHAMNESS GC, JENNINGS AW, and MCGUIRE WL. Estrogen receptor binding to isolated nuclei. A non-saturable process. Biochemistry 13:327-331, 1974.
114. RINGOLD GM. Glucocorticoid regulation of mouse mammary tumor virus gene expression. Biochem Biophys Res Commun 560:487-508, 1979.
115. YAMAMOTO KR, CHANDLER VL, ROSS SR, ET AL. Integration and activity of mammary tumor virus genes: regulation by hormone receptors and chromosomal position. Cold Spring Harbor Symp Quant Biol 45:687-697, 1981.
116. RINGOLD GM, YAMAMOTO KR, BISHOP JM, ET AL. Glucocorticoid-stimulated accumulation of mouse mammary tumor virus RNA: increased rate of synthesis of viral RNA. Proc Natl Acad Sci USA 74:2879-2883, 1977.
117. PAYVAR F, WRANGE O, CARLSTEDT-DUKE J, ET AL. Purified glucocorticoid receptors bind selectively in vitro to a cloned DNA fragment whose transcription is regulated by glucocorticoid in vivo. Proc Natl Acad Sci USA 78:6628-6632, 1981.
118. GOVINDAN MV, SPIESS E, and MAJORS J. Purified glucocorticoid receptor-hormone complex from rat liver cytosol binds specifically to cloned mouse mammary tumor virus long terminal repeats in vitro. Proc Natl Acad Sci USA 79:5157-5161, 1982.
119. PFAHL M. Specific binding of the glucocorticoid-receptor complex to the mouse mammary tumor proviral promoter region. Cell 31:475-482, 1982.

120. MULVIHILL ER, LE PENNEC JP, and CHAMBON P. Chicken oviduct progesterone receptor: location of specific regions of high-affinity binding in cloned DNA fragments of hormone-responsive genes. Cell 24:621-632, 1982.
121. COMPTON JG, SCHRADER WT, and O'MALLEY BW. DNA sequence preference of the progesterone receptor. Proc Natl Acad Sci USA 80:16-20, 1983.
122. HUGHES MR, COMPTON JG, SCHRADER WT, ET AL. Interaction of the chick oviduct progesterone receptor with deoxyribonucleic acid. Biochemistry 20:2481-2491, 1981.
123. WILKS AF, COZENS PJ, MATTAJ IW, ET AL. Estrogen induces a demethylation at the 5′ end region of the chicken vitellogenin gene. Proc Natl Acad Sci USA 79:4252-4255, 1982.
124. MERMOD J-J, BOURGEOIS S, DEFER N, ET AL. Demethylation and expression of murine mammary tumor proviruses in mouse thymoma cell lines. Proc Natl Acad Sci USA 80:110-114, 1983.

Potential Use of Analogs of Luteinizing Hormone-Releasing Hormones in the Treatment of Hormone-Sensitive Neoplasms

Andrew V. Schally,* Tommie W. Redding, and Ana Maria Comaru-Schally[1]

New approaches to the therapy for some endocrine-dependent tumors with analogs of hypothalamic hormones are being developed on the basis of experimental studies in animal models. Analogs of luteinizing hormone-releasing hormones (LH-RH) may open new vistas for the treatment of some hormone-dependent carcinomas. It has been clearly demonstrated that both agonistic and antagonistic analogs of LH-RH can inhibit the growth of rat prostate tumors. A successful treatment of androgen-dependent prostate cancer with agonistic analogs of D-Trp^6-LH-RH, D-Ser(Bu^t)^{6}des-Gly-NH$_2$^{10}-LH-RH ethylamide, and D-Leu^6-des-Gly-NH$_2$^{10}-LH-RH ethylamide has been documented in several hundred patients. The data accumulated so far from clinical trials suggest that LH-RH agonists can be used as an effective endocrine therapy for prostate carcinoma, thereby avoiding the side effects of estrogen and the psychologic impact of castration. Experimental animal studies and some clinical trials suggest that LH-RH agonists and/or antagonists might also be useful in the treatment of breast cancer. The results of experiments with various hypothalamic analogs in animal models of chondrosarcomas, osteosarcomas, and other tumors appear to be encouraging, but the potential clinical efficacy of LH-RH analogs in the treatment of human hormone-sensitive cancers other than breast and prostate remains to be investigated. The approach to treatment of hormone-dependent tumors based on analogs of hypothalamic hormones might become a useful addition to conventional methods for cancer therapy. [Cancer Treat Rep 68:281–289, 1984]

The growth inhibition of endocrine-dependent tumors by analogs of hypothalamic hormones is a relatively recent finding, but it already appears to be of significant clinical value. Ongoing investigations on the application of analogs of luteinizing hormone-releasing hormones (LH-RH) in the field of neoplasms include treatment of breast and prostate cancer, pituitary tumors, chondrosarcomas and osteosarcomas, and other hormone-sensitive tumors. In this review, we shall summarize recent results of experimental and clinical investigations on the effects of agonistic and antagonistic LH-RH analogs on hormone-dependent tumors, with emphasis on the treatment of prostate cancer.

PROSTATE CANCER

Experimental Studies With LH-RH Agonists in Animal Models of Prostate Cancer

LH-RH agonists characterized by a D-amino acid in position 6 were originally developed to treat infertility (1–3). D-Trp^6-LH-RH (fig 1) is a superactive long-acting LH-RH agonist, about 100 times more potent than LH-RH (3). Its effects have been described in > 30 basic and 30 clinical publications (1–11). Although an acute injection of D-Trp^6-LH-RH and other superactive agonists of LH-RH induces a marked and prolonged release of LH and follicle-stimulating hormone (FSH), paradoxically, long-term administration produces dramatic inhibitory effects through a process of "down regulation" of pituitary gonadotrophs and the gonads (1,2,4–13). In male animals, this is manifested by a fall in the weights of testes, seminal vesicles, and prostate, a decrease in plasma testosterone levels, and a reduction in testicular LH/human chorionic gonadotropin (hCG) receptors (2,13,14). In men, a persistent suppression of Leydig cell function, manifested by a fall in serum testosterone, dihydrotestosterone, and estradiol levels, has been observed after long-term administration of D-Trp^6-LH-RH or D-Ser(Bu^t)^{6}des-Gly-NH$_2$^{10}-LH-RH ethyl-

---

[1] Endocrine and Polypeptide Laboratories, Veterans Administration Medical Center, and Section of Experimental Medicine, Department of Medicine, Tulane Medical School, New Orleans, LA.

**Reprint requests to:* Dr. Andrew V. Schally, Veterans Administration Medical Center, Research Bldg 3, 1601 Perdido St, New Orleans, LA 70146.

D-Trp^6-LH-RH

pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2

FIGURE 1.—Molecular structure of D-Trp^6-LH-RH.

amide (HOE 766) (4,15-17).

The finding that prolonged treatment with agonistic analogs of LH-RH can result in testicular inhibition and chemical castration prompted us to use this method in an attempt to induce the regression of prostate tumors in rats. D-Trp^6-LH-RH used in our studies was synthesized by solid phase methods in our laboratory (3) or was supplied by Debiopharm SA, Lausanne, Switzerland. We found that long-term administration of D-Trp^6-LH-RH can inhibit prostate tumor growth in two different rat models: Segaloff 11095 squamous cell carcinoma and Dunning R-3327H adenocarcinoma (7). Administration of D-Trp^6-LH-RH at a dose of 25 μg/day for 14-21 days significantly inhibited the growth of the chemically induced squamous cell carcinoma 11095 in Fisher 344 male rats. The weights of the ventral prostate and testes were also significantly decreased by treatment with this analog. After 21 days of treatment, the animals no longer showed increases in serum LH and FSH levels in response to D-Trp^6-LH-RH. Treatment of male Copenhagen F-1 rats bearing the Dunning R-3327H prostate adenocarcinoma with D-Trp^6-LH-RH (25 μg/day for 42 days) reduced the percent increase in tumor volume to one-third and decreased the actual tumor weight by 58% as compared to untreated controls (7). Tumor cell doubling time was more than four times longer in rats receiving D-Trp^6-LH-RH than in controls. This treatment also diminished the weights of both the ventral prostate and testes, but had no effect on the weight of the anterior pituitary gland. Serum LH and FSH levels were significantly decreased in rats receiving this analog. In both Fisher 344 and Copenhagen F-1 rats, serum prolactin and testosterone levels were significantly reduced after treatment with D-Trp^6-LH-RH, whereas progesterone levels were increased. This study (7) demonstrated for the first time the potential clinical efficacy of D-Trp^6-LH-RH in the treatment of prostate carcinoma and other hormone-sensitive tumors in man.

In a subsequent study (18), rats bearing the hormone-sensitive Dunning R-3327H prostate tumor again showed highly significant reductions in weights of both the ventral prostate and testes after treatment with D-Trp^6-LH-RH for 21 days, but body weights and weights of the adrenal and anterior pituitary glands remained unchanged. Long-term treatment with 25 μg of D-Trp^6-LH-RH twice a day for 21 days induced a highly significant reduction (tenfold) in tumor weight as compared with controls. Tumor volume of these control animals showed a 215% increase, while final tumor volume of the D-TRP^6-LH-RH-treated group was actually smaller by nearly 18% than the initial volume (18). Long-term administration of D-Trp^6-LH-RH significantly reduced LH and prolactin levels, but did not affect growth hormone (GH) values. D-Trp^6-LH-RH greatly suppressed serum testosterone values; the levels were decreased by 93% (18). These two studies (7,18) demonstrated that long-term administration of the agonist D-Trp^6-LH-RH inhibits the growth of Dunning prostate adenocarcinoma R-3327H. The hormone-dependency of this tumor is well-documented (7). In addition, the histologic and biochemical similarities of the Dunning R-3327H prostate tumor to human prostate adenocarcinoma have made this tumor an acceptable model for the study of human prostate cancer (7).

Inhibition of prostate tumor growth in the rat Dunning model by D-Trp^6-LH-RH was accompanied by a significant decrease in serum levels of prolactin and testosterone (7,18). Thus, the principal mechanism of remission of this tumor is probably linked to the suppression of these hormones. The most effective means of suppressing tumor growth in the Dunning rat model is hypophysectomy, either alone or in combination with orchidectomy. The suppressive effect of the LH-RH agonists on gonadotropins, prolactin, and testosterone can mimic, in part, the effect of hypophysectomy and orchiectomy. Prolactin alone or in combination with testosterone has been shown to directly stimulate prostate growth (19). Inhibition of both testosterone and prolactin by long-term administration of LH-RH agonists might thus be expected to suppress tumor growth more effectively than the deficiency of androgen alone. Long-term administration of D-Trp^6-LH-RH and other superagonists of LH-RH may also raise progesterone levels in male rats (7,14,18). It is possible that the high progesterone levels detected in our studies contributed to the growth inhibition of the prostate tumors. A reduction of testicular and ventral prostate weights by D-Trp^6-LH-RH also suggests that superactive LH-RH analogs exert a suppressive effect directly on the testes and that they could antagonize the effects of testosterone (20). In fact, Sundaram et al (20) reported that D-Trp^6-LH-RH ethylamide, which is identical to our agonist except for the presence of an ethylamide group in place of glycine amide in the C-terminal position, could reduce accessory sex organ weights in hypophysectomized male rats maintained on moderate doses of testosterone, and they postulated that LH-RH superagonists could antagonize the action of sex steroids.

It is also possible that D-Trp^6-LH-RH could have some direct inhibitory effect on prostate tumors. LH-RH receptors have been found in the rat testes and ovaries. We have been unable to find LH-RH receptors in normal rat prostate tissue, but we observed binding of D-Trp^6-LH-RH to plasma membranes from Dunning

R-3327H prostate tumors (21). These findings appear to support the view that LH-RH analogs, in addition to their main effect, which is exerted on the pituitary (1,2), and a possible action on the gonads (19), might also act directly on prostatic tumors.

Clinical Studies With LH-RH Agonists in Men With Prostate Cancer

It became immediately apparent that our studies, which demonstrated that D-Trp^6-LH-RH inhibits the growth of prostate tumors in rats, have clinical applications. The first successful palliation of advanced prostatic carcinoma by agonistic analogs of LH-RH was demonstrated in a collaborative trial carried out at the Royal Victoria Hospital in Montreal, Canada (8). Ten patients with prostatic carcinoma (six with stage C and four with stage D disease) were treated from 6 weeks to 12 months with agonistic analogs of LH-RH. Estrogen therapy had been tried unsuccessfully in one patient and was contraindicated for the remaining nine patients because of a history of myocardial infarction or other thromboembolic episodes. D-Trp^6-LH-RH was given sc once daily at a dose of 100 μg, and HOE 766 was given sc at a dose of 50 μg once daily or intranasally at a dose of 500 μg twice daily. In all patients, mean plasma testosterone levels showed a 75% fall by the third week of treatment and remained at castration values thereafter (fig 2). This was followed by a decrease or normalization of serum acid phosphatase levels by the second month of treatment and a $\geqslant$ 47% decrease in serum alkaline phosphatase by the tenth week of treatment in all patients but one, whose tumor was androgen-insensitive. In patients with stage C disease presenting with urinary obstruction, there was noticeable clinical improvement. In two such patients, a decrease in the size of the prostate was confirmed by ultrasonography. In patients with stage D disease manifested by diffuse bone metastases, there was relief of bone pain, and in one patient treated for > 12 months, improvement was documented by radioisotope bone imaging. The only side effects consisted of a decrease in libido and climacteric-like vasomotor phenomena. This trial (8) demonstrated for the first time that superactive agonistic LH-RH analogs may be efficacious therapeutic agents in patients with androgen-sensitive prostatic adenocarcinoma. Furthermore, the study suggests that agonistic analogs of LH-RH may be employed diagnostically to assess the responsiveness of patients to hormonal manipulations. A positive response to LH-RH analogs should help to identify patients bearing hormone-sensitive prostatic adenocarcinomas. Conversely, a lack of response to LH-RH agonists may be useful in detecting patients with androgen-insensitive prostate cancer, to avoid unnecessary surgical castration or estrogen therapy, which is associated with morbidity. Our findings (8) suggested that treatment with agonistic analogs of LH-RH might reduce or eliminate the need for surgical orchiectomy or estrogens in prostate cancer. Jacobi and Wenderoth (16) subsequently pointed out that treatment based on LH-RH agonists might avoid the psychologic impact of castration or even an unnecessary castration, as well as cardiovascular, hepatic, and mammotropic side effects of estrogen. Avoidance of estrogens would prove particularly advantageous for patients at high risk for thromboembolic disease. It is of interest that LH-RH agonists induce tumor regression, despite a concomitant decrease in endogenous estrogen levels during therapy.

Persistent suppression of Leydig cell function, manifested by significant decreases in plasma testosterone and estradiol levels obtained in this study (8) and other studies (4,15-17), suggests that prolonged administration of D-Trp^6-LH-RH or HOE 766 produces "medical" castration. The possibility of a direct effect of LH-RH

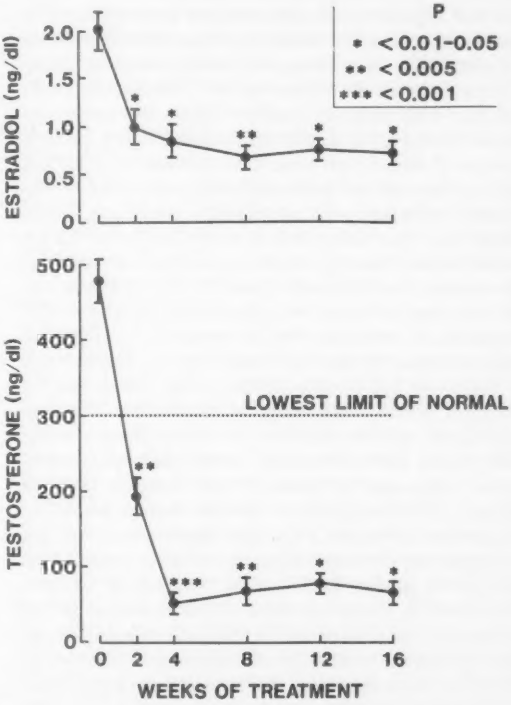


FIGURE 2.—Effects of agonistic analogs of LH-RH on plasma sex steroids in patients with prostatic carcinoma. Note the lowering and persistent suppression of plasma E_2 (upper) and T (lower) during treatment. Dotted line = lowest limit of normal. *P* values: * = < 0.05; ** = < 0.005; *** = < 0.001. From Tolis et al (ref 8), courtesy of the Proceedings of the National Academy of Sciences (USA).

agonists upon human androgen-sensitive prostatic cancer must be considered as well, since our work indicates that rat prostate tumors have receptors for LH-RH (21).

Our findings are being confirmed and extended by other clinical trials in Europe and in North America (16,17,22-27). The Abbott group (22) has reported therapeutic trials at 13 US centers with another agonist of LH-RH, D-Leu^6-des-Gly-NH$_2$^{10}-LH-RH ethylamide (leuprolide). Parenteral administration of leuprolide at doses of 1-20 mg for ≥ 19 weeks to 118 patients with stage D prostatic carcinoma resulted in objective responses, such as a fall in acid phosphatase levels in 60%-70% of the patients. There was a transient flare-up of the disease in about 10% of the patients, but after 4 weeks, androgen values were at castrate levels. The therapy appeared to be effective for prostatic carcinoma, with fewer side effects than those caused by estrogen administration (22).

Borgmann et al (17) treated nine patients with advanced carcinoma of the prostate with HOE 766 and found a fall in testosterone levels after 3 weeks of therapy and regressive changes in some tumors after 3-6 months of treatment, similar to those seen after surgical castration. In a subsequent study, the same group (27) used the HOE 766 analog for 7-19 months in 21 patients with stage C prostate cancer. Seventeen patients showed good therapeutic responses with cytologic signs of tumor regression. Four patients who did not respond because of hormone resistance received secondary treatment with estramustin phosphate. Koutsilieris and Tolis (25) and Tolis et al (26) studied 22 patients with advanced prostatic carcinoma and compared the effects of orchiectomy (Group 1, five patients) versus the effect of long-term administration of HOE 766 (Group 2, 17 patients). Plasma testosterone levels fell more in Group 1 than in Group 2 by the eighth week of follow-up, but became similar in the two groups by the third month. Both therapies resulted in a decrease in prostatic size as estimated by transabdominal ultrasonography, and by the sixth month there was no statistical difference in prostatic size between the two groups. A follow-up of 24 months showed persistent suppression of prostatic size and improvement in signs of prostatism in nearly all of the patients treated with the LH-RH analog (25,26). Bone scanning of patients with stage D$_2$ disease revealed an improvement in five patients in the number and/or intensity of bone lesions. Most patients reported hot flashes and a decrease in libido, but none developed gynecomastia or thromboembolic episodes (25,26).

Labrie et al (23,24) used a combination of HOE 766 with an antiandrogen RU-23908 for treatment of patients with stage C and D$_2$ prostate carcinoma and reported subjective and objective improvement, including a decrease in bone pain and a fall in acid phosphatase.

A significant, sometimes dramatic decrease in bone pain in patients with stage D metastatic prostate carcinoma after treatment with agonists of LH-RH is a frequent and consistent finding (8,16-18,22-27).[2,3,4] A decrease in the number and intensity of bone lesions has also been reported (8,16-18,22-27).[2,3,4] Mathé et al[3] used D-Trp^6-LH-RH to treat 21 patients with stage D, three with stage C, and one with stage B prostatic carcinoma. After 3 months of therapy, 84% of the patients showed a normalization or decrease of tumor mass (measured by ultrasonography), and an improvement in bone metastases (evaluated by bone scan) was observed in 44% of the patients. The levels of prostatic acid phosphatase decreased in 75% of the patients. Nineteen of 21 patients showed a total disappearance or improvement in bone pain. No side effects, except for hot flashes and decrease in potency, were observed.

Thus, data are accumulating which suggest that long-term therapy with D-Trp^6-LH-RH and other agonists of LH-RH might be the preferred alternative to surgical castration or treatment with estrogens. The regression of prostatic tumors and metastatic disease manifested clinically, radiologically, biochemically, and cytologically after treatment with LH-RH agonists is most encouraging (8,17,18,22-27). However, more time is needed to assess the duration of response to LH-RH agonists and the effect on survival rate.

It is also likely that treatment with LH-RH agonists can be combined with chemotherapy. Thus, simultaneous administration of one of the cytotoxic drugs, probably cyclophosphamide, and D-Trp^6-LH-RH or other LH-RH agonists, early in the diagnosis of metastatic prostate cancer, could inhibit the proliferation of androgen-independent cell clones, and therefore enhance the therapeutic response. Such a combination of chemotherapy and hormonal approach has already been tried with other agents by Murphy et al (28).

It should also be mentioned that some agonists, such as D-Trp^6-LH-RH and HOE 766, are available not only as preparations for parenteral administration, but also as intranasal sprays. In addition, future treatment of prostate carcinoma should be made more convenient by the development of injectable microcapsules with D-Trp^6-LH-RH for once-a-month administration (18). These microcapsules, prepared from a biodegradable biocompatible polymer system (poly[d,1-lactide-co-glycolide]), are designed for a constant, controlled release of this analog over a 30-day period. The microencapsulated D-Trp^6-LH-RH is expected to be efficacious in hu-

---

[2] Gonzalez-Barcena D, Perez PL, Berea H, et al. Presented at the 65th Annual Meeting of the Endocrine Society, San Antonio, TX, June 8-10, 1983.

[3] Mathé G, Vo Van ML, Duchier J, et al. Presented at the del Duca Foundation Symposium on Tumor Pharmacotherapy, Paris, June 23, 1983.

[4] Kaplan E. Unpublished personal communication.

mans, and is thus expected to increase the utility of this analog of LH-RH for treatment of prostate carcinoma and other hormone-sensitive neoplasms.

Experimental Studies With LH-RH Antagonists on Prostate Tumors in Rats

Antagonistic analogs of LH-RH were originally developed for contraception (1,2). These analogs, which possess modifications in positions 2, 3, and 6, and in more recent compounds other changes, especially in positions 1 and 10, act on the same receptor sites as LH-RH and inhibit the release of gonadotropins. A series of potent LH-RH antagonists have been synthesized and tested in a number of animal species and in humans (1,2). In women and subhuman primates, these antagonists disrupt the normal events of the menstrual cycle, block ovulation, and inhibit the rise in LH and FSH induced by oophorectomy (1,2). In male rats, administration of LH-RH antagonists has been shown to decrease testosterone production and to reduce the weights of testes and accessory sex organs (29). An early antagonistic analog blocked the LH and FSH responses to LH-RH in men for at least 24 hours (1,2,30).

Successful suppression of Segaloff and Dunning prostate tumors by long-term administration of the agonist D-Trp^{6}-LH-RH (7) prompted us to investigate whether inhibitory analogs would also have an effect. So far, three antagonistic analogs have been used in our studies on rat models of prostate tumors (29). Antagonists [NAc-D-p-F-Phe^{1},p-Cl-D-Phe^{2},D-Trp^{3,6},D-Ala^{10}]LH-RH (Antagonist 1), [NAc-D-p-Cl-Phe^{1,2},D-Trp^{3},D-Phe^{6},D-Ala^{10}]LH-RH (Antagonist 2), and N-Ac-D-p-Cl-Phe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}-LH-RH (Antagonist 3) were prepared by solid-phase methods in our laboratory (31,32) or by classical synthesis by NV Organon, Oss, Netherlands. First, we investigated the effects of Antagonist 1 on the growth of the chemically induced Segaloff 11095 squamous cell carcinoma in Fisher 344 male rats (29). Long-term administration of this antagonist at doses of 50 μg/day for 21 days significantly reduced tumor weight as compared with controls, but the weights of the pituitary, ventral prostate, and testes were not significantly altered. After 21 days of treatment with this analog, serum LH, FSH, and testosterone levels were markedly decreased. When male Copenhagen F-1 rats bearing the Dunning R-3327H prostate adenocarcinoma were injected with Antagonist 1 or with Antagonist 2 at doses of 50 μg/day, the percent increase in tumor volume was decreased to one-half or less, and actual tumor volume was decreased by 34%–96% compared to controls (29). Tumor weight was decreased by 30% and 89% after Antagonists 1 and 2, respectively, as compared to untreated controls. Tumor doubling time was threefold to fourfold longer in rats receiving inhibitory analogs than in controls. Treatment with Antagonist 2 decreased the weight of the whole prostate, but neither antagonist changed the weight of testes, anterior pituitary gland, or adrenals (29). Serum LH, FSH, and testosterone levels in Copenhagen F-1 rats bearing Dunning tumors were significantly decreased after treatment with the inhibitory analogs, but progesterone levels were increased (29).

In the second study (18), we used a more modern antagonist (No. 3) (fig 3), which is considerably more potent in blocking ovulation than analogs used previously and active when administered orally to rats (32). In addition, this antagonist has more desirable solubility properties, being soluble in water and saline (32). Administration of Antagonist 3 at a dose of 25 μg twice a day for 3 weeks also significantly inhibited growth of the Dunning prostate tumor. Mean tumor weight in the antagonist-treated group at autopsy was only 0.38 ± 0.05 g, as compared with 8.55 ± 4.6 g for controls. Tumor volumes showed only a small increase (13.7%) during the 3-week treatment with the antagonist, in contrast to a 215% change in the control group (18). Treatment with the LH-RH antagonist did not significantly affect body or testes weights. However, the adrenal, anterior pituitary, and ventral prostate showed significant decreases in weights. Long-term administration greatly suppressed serum testosterone values; the levels were decreased by 97% (18). The antagonist also significantly reduced LH and prolactin levels, but did not affect GH values (18).

The inhibition of prostate carcinomas by antagonistic LH-RH analogs is probably mainly due to the suppression of plasma levels of testosterone and prolactin (29,18), and is similar to that achieved by long-term administration of LH-RH agonists. The binding of a powerful antagonist to plasma membranes from Dunning R-3327H prostate tumors indicates that LH-RH antagonists might also act directly on prostatic tumors (21).

These experimental studies, which showed an inhibition of growth of prostate tumors in rats after administration of three different LH-RH antagonists (18,29), suggest that this class of compounds could also be used for the treatment of prostate carcinoma.

The use of antagonistic analogs of LH-RH for the treatment of prostate cancer would avoid the transient stimulation of the release of gonadotropins and testosterone which occurs initially in response to LH-RH agonists, thus preventing the temporary clinical flare-up of the disease (18). However, LH-RH antagonists have been tried so far only in short-term studies in humans aimed at contraception (1,2), and clinical data are not yet available on the effects of long-term adminis-

(N-Ac-D-p-Cl-Phe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}) -LH-RH

FIGURE 3.—Molecular structure of the D-Arg^{6} antagonistic analog.

tration. Large-scale synthesis and completion of toxicologic investigations should permit (*a*) clinical evaluation of LH-RH antagonists in the treatment of prostate cancer and (*b*) comparison of their therapeutic efficacy with D-Trp^6-LH-RH and other agonists.

BREAST CANCER

Experimental Studies With LH-RH Agonists on Mammary Tumors in Rats and Mice

Long-term administration of superactive agonistic analogs of LH-RH induces paradoxical antifertility effects in female rats analogous to those reported in males (1,2,5,6,12). Among the effects reported are: delay in vaginal opening, reduction in ovarian and uterine weight, interference with mating and implantation, termination of gestation, and a fall in ovarian receptors for LH/hCG (1,2,5,6,12). These antifertility effects, which occur in response to treatment with LH-RH agonists, have been observed in females of other species, including primate and human (33), but not in mice (34). Various groups have demonstrated a regression in the growth of DMBA-induced mammary carcinoma in rats after administration of several superactive agonists (35-39).

We have used D-Trp^6-LH-RH in rat and mouse models of endocrine-dependent mammary tumors (9). Tumor weights in BDF$_1$ mice bearing the MXT 3.2 mammary adenocarcinoma were significantly decreased after administration of 25 μg/day of D-Trp^6-LH-RH for 21 days (9). Final tumor volume was also reduced by 47%. Ovarian weights were decreased by only 22%, but final body weights were not changed from those of controls. Treatment with D-Trp^6-LH-RH lowered plasma progesterone, but not to ovariectomy levels (9). Since mice are reputedly refractory to inhibitory effects of LH-RH agonists (34), it is possible that D-Trp^6-LH-RH induced mammary tumor regression by some other mechanism.

In Wistar-Furth rats bearing the MT/W9A mammary adenocarcinoma, treatment with 25 μg of D-Trp^6-LH-RH twice a day for 28 days significantly decreased tumor weights by > 50% and tumor volume by 67% (9). The percent change in tumor volume (-51%), based on individual responses, was also highly significant when compared to controls. Ovarian weights decreased by 38% and anterior pituitary weights were reduced by 21%, but final body weights remained unchanged (9). A small number of rats were ovariectomized after transplantation of the tumor to test for estrogen dependency of the carcinoma. After 28 days, tumor weights were decreased by > 90% and, in some rats, the tumor had disappeared.

In rats bearing MT/W9A tumors, administration of D-Trp^6-LH-RH for 28 days significantly decreased progesterone levels by 74% and estradiol by 42%. Serum LH did not fall appreciably, but prolactin levels were suppressed by 70%, as compared to control rats (9).

The estrogen-dependent MT/W9A mammary tumor requires physiologic levels of prolactin for its growth. Progesterone may also have a primarily stimulatory role. Regression of this tumor may be due mainly to the suppressive effects of D-Trp^6-LH-RH on estrogen and progesterone levels (9) and is in agreement with results obtained with other agonists of LH-RH in the DMBA rat model (35-39). A direct action of the agonist D-Trp^6-LH-RH on tumor tissue itself also cannot be ruled out. However, it has not been determined whether LH-RH-like receptors exist in mammary tumor tissue. The regression of mammary tumors in rats and mice after long-term administration of agonistic analogs of LH-RH suggests that they could be tried for treatment of breast carcinoma in premenopausal women.

Experimental Studies With LH-RH Antagonists on Mammary Tumors in Rats and Mice

Two powerful analogs of LH-RH, Antagonists 4 (N-Ac-D-p-Cl-Phe^{1,2},Phe^3,D-Arg^6,D-Ala^{10}-LH-RH) (31) and 3 (32), were also investigated by us in rat and mouse models of endocrine-dependent mammary tumors (9). Administration of 25 μg/day of Antagonist 4 for 3 weeks significantly reduced the weight and volume of estrogen-dependent MXT 3.2 mammary tumors in BDF$_1$ mice. Ovarian weights and plasma levels of progesterone were also diminished after treatment with this antagonist. Antagonist 3, administered for 4 weeks at doses of 50 μg/day to Wistar-Furth rats, reduced the weight and volume of MT/W9A mammary tumor by 58% and 42%, respectively (9). The percent change in tumor volume (-40%) was similarly highly significant after treatment with the antagonist. LH, estrogen, and progesterone levels were also greatly reduced in rats treated with the antagonist. Suppression of tumor growth by the antagonists is most likely linked with the inhibition of the levels of sex steroids, but some direct action of the antagonists on mammary tumors cannot be excluded. Inhibition of mammary carcinoma in animals by administration of D-Trp^6-LH-RH or inhibitory analogs of LH-RH and the investigation of the molecular basis of this phenomenon should shed more light on the endocrine and cellular mechanisms connected with these neoplasms. The regression of mammary tumors in rats and mice in response to inhibitory analogs of LH-RH (9) represents novel and possibly important findings and may lead to development of a new type of therapy for breast carcinoma in women based on these antagonists.

Clinical Studies With LH-RH Agonists in Women With Breast Carcinoma

The response of postmenopausal women with metastatic breast cancer to therapy with LH-RH agonists has not been impressive (40,41, and unpublished reports by various groups). Nevertheless, occasional responses have been recorded. Recently, Mathé and Vo Van (personal communication) reported 70% tumor regression and disappearance of the axillary lymph node metastases in a postmenopausal patient treated with D-Trp^6-LH-RH for 3 months. This patient had diagnosis of bilateral breast cancer with pulmonary metastases and was previously treated with local irradiation and tamoxifen, but had a relapse.

On the basis of our experimental work, we felt that premenopausal patients with breast cancer should respond better to the LH-RH analogs than postmenopausal patients. Recent preliminary results are in line with this view. Klijn and DeJong (41) reported that two of four premenopausal women with metastatic breast cancer improved after 7 weeks of treatment with HOE 766. In another study, in which the leuprolide analog of LH-RH was used, some clinical improvement in about 40% of premenopausal women with breast cancer was also detected (40).

It is possible that antagonistic analogs of LH-RH may be superior in this respect to agonists, and thus lead to greater regression of breast cancer in premenopausal and perhaps even postmenopausal patients. The same considerations as those discussed above in the section on prostate cancer regarding combined use of LH-RH analogs with chemotherapy could also apply to breast cancer. Such combined therapy might lead to an increase in the therapeutic response. Development of a new method of therapy for breast cancer in women based on agonistic or antagonistic analogs of LH-RH might reduce the need for mastectomies and/or increase the survival rate of some patients with hormone-sensitive tumors.

Experimental Studies With LH-RH Agonists and Antagonists in Pituitary Tumors

Lamberts et al (42) recently showed that long-term administration of an LH-RH agonist inhibited the growth of estrogen-dependent prolactin- and adrenocorticotropic-hormone-secreting pituitary tumor 7315a in rats. The results of our studies also demonstrate that daily administration of D-Trp^6-LH-RH (at a dose of 25 μg/day for 14–24 days to female Buffalo rats, starting 3–18 days after inoculation with tumor 7315a) reduced its weight and volume (11). Antagonist 2 also significantly inhibited growth of the pituitary tumor (11). Serum prolactin levels were significantly reduced in the groups treated with D-Trp^6-LH-RH or the antagonist (11). The mechanism by which D-Trp^6-LH-RH or the antagonist inhibits the growth of the PRL-secreting rat pituitary tumor may be related to a suppression of sex steroid levels (11). It is possible that these findings could be of some clinical significance. It cannot be excluded that therapy based on analogs of LH-RH alone or combined with somatostatin analogs could be of value in the treatment of patients with pituitary tumors who failed to respond to surgery, irradiation, and bromocriptine treatment.

Experimental Studies With Murine Chondrosarcomas (Rat) and Osteosarcomas (Mouse)

It has been established that, as in the case of normal cartilage, chondrosarcoma chondrocytes are hormone-dependent, being influenced by GH or somatomedins, insulin, and glucocorticoids (43,44). We have investigated the effect of D-Trp^6-LH-RH and some analogs of somatostatin on the growth of Swarm chondrosarcoma (10).

In two experiments, long-term administration of D-Trp^6-LH-RH at a dose of 30–60 μg/day for 22–30 days to rats bearing transplantable Swarm chondrosarcoma caused a significant reduction in tumor weight and/or volume. D-Trp^6-LH-RH, given together with pNH$_2$Phe^4-Somatostatin, also caused a highly significant decrease in tumor weight.

In another experiment, D-Trp^6-LH-RH, alone or in combination with another analog, D-5F-Trp^8-Somatostatin, reduced tumor volume after 14 days of treatment (10). Serum levels of GH and prolactin in rats bearing the Swarm chondrosarcoma were significantly decreased in rats treated with D-Trp^6-LH-RH. There appeared to be a greater suppression of GH and prolactin levels when D-5F-Trp^8-Somatostatin was administered together with D-Trp^6-LH-RH (10). We have also recently determined that D-Trp^6-LH-RH and some analogs of somatostatin exert similar inhibitory effects on the growth of mouse osteosarcomas and significantly extend survival rates.

While the growth inhibition of chondrosarcomas and osteosarcomas by analogs of somatostatin could be explained by their suppressive effects on circulating GH and insulin levels, the mechanism of suppression of these tumors by D-Trp^6-LH-RH is not clear.

These observations that long-term administration of analogs of somatostatin and D-Trp^6-LH-RH inhibit the growth of the rat chondrosarcoma and mouse osteosarcoma could be of clinical importance (10). In patients with osteogenic malignancies where conventional therapy has failed, treatment with analogs of somatostatin alone or combined with D-Trp^6-LH-RH could perhaps be of value. In any event, the growth inhibition of the Swarm chondrosarcoma in rats (10) and the prolongation of survival rates of mice with osteosarcomas by analogs of hypothalamic hormones raise some hope that, eventually, such analogs might lead to a new en-

docrine therapy for chondrosarcomas, osteosarcomas, and related hormone-dependent neoplasms.

CONCLUSION

A new approach to the treatment of various endocrine-dependent tumors based on agonists and antagonists of LH-RH and other analogs of hypothalamic hormones is still in the early stages of development, but appears to be promising. The collective data from experimental work with animal tumor models and some clinical trials support the contention that the use of hypothalamic analogs might supplement and, in some cases, replace conventional procedures for the treatment of hormone-sensitive cancers.

REFERENCES

1. SCHALLY AV, ARIMURA A, and COY DH. Recent approaches to fertility control based on derivatives of LH-RH. *In* Vitamins and Hormones (Munson PL, Glover E, Diczfalusy E, et al, eds). New York, Academic Press, 1980, vol 38, pp 257-323.
2. SCHALLY AV, COY DH, and ARIMURA A. LH-RH agonists and antagonists. Int J Gynaecol Obstet 18:318-324, 1980.
3. COY DH, VILCHEZ-MARTINEZ JA, COY EJ, ET AL. Analogs of luteinizing hormone-releasing hormone with increased biological activity produced by D-amino acid substitutions in position 6. J Med Chem 119:423-425, 1976.
4. TOLIS G, MEHTA A, COMARU-SCHALLY AM, ET AL. Suppression of androgen production by D-Tryptophan-6-LH-RH in man. J Clin Invest 68:819-822, 1981.
5. VILCHEZ-MARTINEZ JA, PEDROZA E, ARIMURA A, ET AL. Paradoxical effects of D-Trp^6-luteinizing hormone-releasing hormone on the hypothalamic-pituitary-gonadal axis in immature female rats. Fertil Steril 31:677-682, 1979.
6. ARIMURA A, PEDROZA E, VILCHEZ-MARTINEZ JA, ET AL. Prevention of implantation by D-Trp^6-LH-RH in the rat: comparative study with the effects of large doses of hCG on pregnancy. Endocr Res Commun 4:357-366, 1977.
7. REDDING TW, and SCHALLY AV. Inhibition of prostate tumor growth in two rat models by chronic administration of D-Trp^6-LH-RH. Proc Natl Acad Sci USA 78:6509-6512, 1981.
8. TOLIS G, ACKMAN A, STELLOS A, ET AL. Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing agonists. Proc Natl Acad Sci USA 79:1658-1662, 1982.
9. REDDING TW, and SCHALLY AV. Inhibition of mammary tumor growth in rats and mice by administration of agonistic and antagonistic analogs of luteinizing hormone-releasing hormone. Proc Natl Acad Sci USA 80:1459-1462, 1983.
10. ———. Inhibition of growth of the transplantable rat chondrosarcoma by analogs of hypothalamic hormones. Proc Natl Acad Sci USA 80:1078-1082, 1983.
11. DE QUIJADA MG, REDDING TW, COY DH, ET AL. Inhibition of the growth of a prolactin-secreting pituitary tumor in rats by analogs of luteinizing hormone-releasing hormone and somatostatin. Proc Natl Acad Sci USA 80:3485-3488, 1983.
12. CORBIN A, BEATTIE CW, TRACY J, ET AL. The anti-reproductive pharmacology of LH-RH and agonistic analogues. Int J Fertil 23:81-92, 1978.
13. SANDOW J, VON RECHENBERG W, JERZABEK G, ET AL. Pituitary gonadotropin inhibition by a highly active analog of luteinizing hormone-releasing hormone. Fertil Steril 30:205-209, 1978.
14. AUCLAIR C, KELLY PA, COY DH, ET AL. Potent inhibitory activity of (D-Leu^6,Des-Gly-NH_2)LH-RH ethylamide on LH/hCG and PRL testicular receptor levels in the rat. Endocrinology 101:1890-1893, 1977.
15. BERGQUIST C, NILLIUS SJ, BERGH T, ET AL. Inhibitory effects on gonadotropin secretion and gonadal function in men during chronic treatment with a potent stimulatory luteinizing hormone-releasing hormone analogue. Acta Endocrinol 91:601-608, 1979.
16. JACOBI GH, and WENDEROTH UK. Gonadotropin-releasing hormone analogues for prostate cancer: untoward side effects of high-dose regimens acquire a therapeutical dimension. Eur Urol 8:129-134, 1982.
17. BORGMANN V, HARDT W, SCHMIDT-GOLLWITZER M, ET AL. Sustained suppression of testosterone production by the luteinizing hormone-releasing hormone agonist buserelin in patients with advanced prostate carcinoma. Lancet 1:1097-1099, 1982.
18. SCHALLY AV, REDDING TW, and COMARU-SCHALLY AM. Inhibition of prostate tumors by agonistic and antagonistic analogs of LH-RH. Prostate 4:545-552, 1983.
19. GRAYHACK JT. Pituitary factors influencing growth of the prostate. Natl Cancer Inst Monogr 12:189-199, 1962.
20. SUNDARAM K, CAO YQ, WANG N, ET AL. Inhibition of the action of sex steroids by gonadotropin-releasing hormone (GnRH) agonists: a new biological effect. Life Sci 28:83-88, 1981.
21. HIEROWSKI MT, ALTAMIRANO P, REDDING TW, ET AL. The presence of LH-RH-like receptors in Dunning R-3327H prostate tumors. FEBS Lett 154:92-96, 1983.
22. GLODE LM. Leuprolide therapy of advanced prostatic cancer. *In* Proceedings of the 18th Annual Meeting of the American Society of Clinical Oncology, Apr 25-27, 1982, p 110.
23. LABRIE F, DUPONT A, BELANGER A, ET AL. New hormonal therapy in prostatic carcinoma: combined treatment with an LH-RH agonist and an antiandrogen. J Clin Invest Med 5:267-275, 1982.
24. ———. New approach in the treatment of prostate cancer: complete instead of only partial removal of androgens. Prostate 4:579-594, 1983.
25. KOUTSILIERIS M, and TOLIS G. Gonadotropin releasing hormone agonistic analogues in the treatment of advanced prostatic carcinoma. Prostate 4:569-577, 1983.
26. TOLIS G, KOUTSILIERIS M, FAZEKAS ATA, ET AL. Transabdominal ultrasonography in the evaluation of patients with advanced prostatic carcinoma: effects of castration and of chronic administration of a gonadotropin releasing hormone agonistic analogue. Prostate 4:459-600, 1983.
27. BORGMANN V, NAGEL R, AL-ABADI H, ET AL. Treatment of prostatic cancer with LH-RH analogues. Prostate 4:553-568, 1983.
28. MURPHY GP, SUNMOLU B, BRADY MF, ET AL. Treatment of newly diagnosed metastatic prostatic cancer patients with chemotherapy agents in combination with hormones versus hormones alone. Cancer 51:1264-1272, 1983.
29. REDDING TW, COY DH, and SCHALLY AV. Prostate carcinoma tumor size in rats decreases after administration of antagonists of luteinizing hormone-releasing hormone. Proc Natl Acad Sci USA 79:1273-1276, 1982.
30. GONZALEZ-BARCENA D, COY DH, KASTIN AJ, ET AL. Suppression of gonadotrophin release in man by an inhibitory analogue of LH-releasing hormone. Lancet 2:997-998, 1977.
31. ERCHEGYI J, COY DH, NEKOLA MV, ET AL. Luteinizing normone-releasing hormone analogs with increased anti-ovulatory activity. Biochem Biophys Res Commun 100:915-920, 1981.
32. COY DH, HORVATH A, NEKOLA MV, ET AL. Peptide antagonists of LH-RH: large increases in antiovulatory activities produced by basic D-amino acids in the six position. Endocrinology 110:1445-1447, 1982.
33. BERGQUIST C, NILLIUS SJ, and WIDE L. Intranasal gonadotropin-releasing hormone agonist as a contraceptive agent. Lancet 2:215-216, 1979.
34. BEX FJ, CORBIN A, and FRANCE E. Resistance of the mouse to the antifertility effects of LH-RH agonists. Life Sci 30:1263-1269, 1982.

35. DESOMBRE ER, JOHNSON ES, and WHITE WF. Regression of rat mammary tumors effected by a gonadoliberin analog. Cancer Res 36:3830–3833, 1976.
36. JOHNSON ES, SEELY JH, and WHITE WF. Endocrine-dependent rat mammary tumor regression: use of a gonadotropin releasing hormone analog. Science 194:329–330, 1976.
37. ROSE DP, and PRUITT B. Modification of the effect of a gonadoliberin analog on 7,12-dimethylbenz(*a*)anthracene-induced rat mammary tumors by hormone replacement. Cancer Res 39:3968–3970, 1979.
38. NICHOLSON RI, and MAYNARD PV. Anti-tumor activity of ICI 118630, a new potent luteinizing hormone-releasing hormone agonist. Br J Cancer 39:268–273, 1979.
39. DANGUY A, LEGROS N, HEUSON-STIENNON JA, ET AL. Effects of a gonadotropin-releasing hormone (GnRH) analogue (A-43818) on 7,12-dimethylbenz(*a*)anthracene-induced rat mammary tumors. Histological and endocrine studies. Eur J Cancer Clin Oncol 13: 1089–1094, 1977.
40. HARVEY HA, LIPTON A, and MAX D. LH-RH analogs in the treatment of human breast cancer. *In* LH-RH and Its Analogs—A New Class of Contraceptive and Therapeutic Agents (Vickery BH, Nestor JJ, Jr, and Hafez ESE, eds). Lancaster, MTP Press, 1984.
41. KLIJN JGM, and DEJONG FH. Treatment with a luteinizing-hormone releasing hormone analogue (buserelin) in premenopausal patients with metastatic breast cancer. Lancet 1:1213–1216, 1982.
42. LAMBERTS SWJ, UITTERLINDEN P, ZUIDERWIZK-ROEST JM, ET AL. Effects of a luteinizing hormone-releasing hormone analog and tamoxifen on the growth of an estrogen-induced prolactin-secreting rat pituitary tumor and its influence on pituitary gonadotropins. Endocrinology 108:1878–1884, 1981.
43. SALOMON DS, PAGLIA LM, and VERBRUGGEN L. Hormone-dependent growth of a rat chondrosarcoma in vivo. Cancer Res 39:4387–4395, 1979.
44. MCCUMBEE WD, MCCARTY KS, JR, and LEBOVITZ HE. Hormone responsiveness of a transplantable rat chondrosarcoma. II. Evidence for in vivo hormone dependence. Endocrinology 106: 1930–1940, 1980.

Promise of Radiosensitizers and Radioprotectors in the Treatment of Human Cancer[1]

Theodore L. Phillips[2,*] and Todd H. Wasserman[3,4]

Over the past 30 years, as an understanding of radiation chemistry has developed, it has been possible to develop compounds which modify the initial radiochemical event. In addition, certain physiologic means such as hyperbaric oxygen or blood flow restriction have also been developed as methods to modify radiation response through the radiochemical processes. Following the success of certain hyperbaric trials, a great effort was made to develop chemical agents which would mimic oxygen in their sensitization of hypoxic cells. A large series of compounds have now been identified with such properties and several of these have entered clinical trial. The first compound to receive widespread testing, misonidazole, has proven too toxic to be used in adequate doses for clinically relevant sensitization. Two new nitroimidazole analogs, which are excluded from the central nervous system, promise to allow much higher degrees of sensitization in planned clinical trials and new non-nitro drugs are under development. Radioprotection can be achieved through various methods, including restriction in blood flow and the use of sulfhydryl-containing compounds which again modify the initial radiochemical events. To be successful in tumor therapy, such agents must be selective in protecting the normal tissues. One class of compounds, the thiophosphates, show differential protection of normal tissue vis-à-vis tumor through several mechanisms. After extensive animal testing, one of these compounds, WR 2721, is now in phase I clinical testing, with phase II evaluation planned for the near future. Other potential sensitizers with varying degrees of differential activity in tumor versus normal tissue are also discussed. [Cancer Treat Rep 68:291–302, 1984]

The recognition by Gray and his colleagues (1) in 1953 that oxygen had a major effect on radiosensitivity has led to extensive investigations into the potential for this factor in tumor therapy. Numerous pathologic studies revealed the presence of necrotic zones in human tumors and extensive animal testing has proven the presence of 1%–30% hypoxic cells in most solid tumors. This knowledge led to several trials of hyperbaric oxygen, in order to increase the access of oxygen to the hypoxic and more radioresistant cells, and some of the trials showed positive results. In particular, trials in head and neck cancer and those in carcinoma of the cervix showed a benefit for patients treated in the presence of high-pressure oxygen (2,3).

This initial success was tempered by the cumbersome nature of hyperbaric oxygen administration and for the past 20 years an extensive search has been conducted for compounds which would interact with basic radiochemical events and/or modify the development or repair of radiation injury in a differential fashion (4).

Other methods of differential sensitization such as incorporation of analogs into DNA in rapidly dividing tumors, modification of naturally occurring radioprotectors, and interference with repair of certain kinds of radiation injury have also received extensive laboratory but as yet little clinical evaluation.

Differential protection rather than sensitization has also been promised by the further understanding of radio-

---

[1] Supported by Public Health Service grants CA-21439 and CA-10261 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[2] Department of Radiation Oncology, University of California—San Francisco.

[3] Division of Radiation Oncology, Washington University School of Medicine, St. Louis, MO.

[4] We thank the members of the Radiation Therapy Oncology Group and those who worked on the Research Plan for Radiation Oncology for their help in obtaining data for this paper, and Cynthia L. Vegas for preparing this manuscript.

[*] *Reprint requests to:* Theodore L. Phillips, MD, L-75, Department of Radiation Oncology, University of California San Francisco, San Francisco, CA 94143.

chemical events and by the development of highly hydrophilic protective compounds which are now in early clinical testing (5). This paper will discuss the various types of radiosensitizers and protectors, the rationale for their use, the hypotheses to be tested in the clinic, and the results to date of clinical studies.

RADIOSENSITIZERS

Rationale

There are currently at least five classes of compounds with potential for differential sensitization of tumor versus normal tissue. There needs to be a definite biologic reason for a compound to cause greater enhancement of radiation injury in tumor vis-à-vis normal tissue or there would be no benefit to the therapeutic ratio in the use of such an agent. The first rationale used in the application of differential sensitization was the discovery of hypoxic cells within tumors approximately 30 years ago (1,6). It is now widely recognized that cells in rapidly growing tumors become deprived of oxygen through the abnormality of the tumor blood supply and the rapidity of tumor cell growth versus capillary proliferation. Limitations in oxygen diffusion distance compound this problem and lead to the presence of 1%-30% hypoxic cells in most solid rodent tumors. Table 1 shows the various methods of modifying the sensitivity of hypoxic cells and the degree to which the agents have had laboratory and clinical testing. Oxygen was the first radiation sensitizer employed and led to positive clinical results in the head and neck and cervix trials conducted in the United Kingdom (2,3). The addition of oxygen in the form of increased ambient pressure causes further oxygen solution in the blood and deeper penetration into tumor cords. The sensitization of animal tumors and most likely human tumors is variable, however, because of the physiologic changes that exposure to hyperbaric oxygen causes in tumor blood flow. Thus, hyperbaric oxygen may not be universally active from patient to patient or from tumor type to tumor type.

The difficulties encountered in administering hyperbaric oxygen and the potential physiologic problems led to an extensive laboratory effort to develop chemical sensitizers which would mimic oxygen. Adams and Dewel (4) and, later, others realized that the initial lesion caused in the DNA contained free radicals. These free radicals can be either repaired through interaction with intracellular sulfhydryl groups or fixed through electron transfer to oxygen or other electron-affinic compounds. This competitive reaction between endogenous sulfhydryls or sulfhydryls added as a protective chemical and oxygen or other electron-affinic compounds can change the sensitivity of these cells by a factor of up to 3.

The first group of compounds which proved to be sufficiently nontoxic for in vivo use and which sensitized hypoxic mammalian cells were the nitroimidazoles. These compounds have now received extensive clinical testing throughout the world (7). Because the earlier compounds introduced for clinical testing proved to be highly toxic to the peripheral (PNS) and even central nervous system (CNS), there has been extensive synthesis work and laboratory as well as clinical testing to develop less neurotoxic compounds. These compounds will be discussed below.

Further synthesis work which has not yet reached the clinic is attempting to develop nitroimidazole alkylating-agent combinations, which are more potent as sensitizers with perhaps less general toxicity, and non-nitro-containing compounds, which may not show neurotoxicity and could be combined with the nitro compounds for greater sensitization (8). Other work is being carried out with blood-replacement compounds, such as the perfluoro chemicals, which can carry large amounts of oxygen and yet be packaged in very small particles capable of penetrating through very narrow vessels and which will release oxygen at very low oxygen tensions (9). These compounds are not chemical sensitizers but could carry increased oxygen to hypoxic tumor zones and be useful clinically. They are only in the earliest stages of laboratory study as potential radiosensitizers but have been used in the clinic for other conditions (10).

In addition to the activity of nitroimidazoles as hypoxic cell sensitizers, they have been shown to develop other properties after prolonged incubation under hypoxic conditions. Such nitroimidazole compounds cause direct cytotoxicity to hypoxic cells and a reduction in the shoulder of the radiation survival curve. These effects have been shown both in vitro and in vivo but have not yet been demonstrated convincingly as occurring in clinical trials to date (11). These effects of nitroimidazoles are interest-

TABLE 1.—Hypoxic cell sensitizers

	In vitro	In vivo	Types of human studies
Oxygen	Yes	Yes	Phase I-III, positive
Perfluorochemicals	—	—	—
Nitroimidazoles	Yes	Yes	Phase I-III, equivocal
Nitroimidazole/alkylators	Yes	Yes	—
Non-nitro agents	Yes	—	—
Preincubation effect of nitroimidazole	Yes	Yes	—

TABLE 2.—Thiol-depleting agents

	In vitro	In vivo	Types of human studies
Diamide	Yes	No	—
N-Ethylmaleimide	Yes	Yes	—
Diethylmaleate	Yes	Yes	—
Dimethylfumarate	Yes	Yes	—
Buthione-SR-sulfoximine	Yes	Yes	—

ing, since removal of the shoulder on the radiation survival curve for hypoxic cells could cause enhancement of cell killing during fractionated radiotherapy. In addition, agents cytotoxic only to hypoxic cells could augment cancer chemotherapeutic agents. Synthesis programs to exploit these properties of nitroimidazoles are needed.

The second class of agents for which a rationale exists as potential preferential tumor sensitizers are the thiol-depleting agents shown in table 2. All cells contain varying degrees of non-protein sulfhydryls which act to protect the DNA. As the level of these compounds is reduced to low concentrations either through chemical suppressors such as *N*-ethylmaleimide or through synthesis blockers such as buthione-SR-sulfoximine, sensitization of the cell occurs (12). In addition to this effect, the depletion of thiol enhances the sensitizing efficiency of nitroimidazole hypoxic cell sensitizers (13). Therefore, these agents have the potential to sensitize cells on their own and to sensitize hypoxic cells to a greater degree than well-aerated cells. They also have the interesting property of enhancing the effectiveness of existing nitroimidazole sensitizers. If this were to occur without increased neurotoxicity, they might have great clinical usefulness.

The third area of sensitizer application with differential potential involves pyrimidine analogs. The original finding that substitution of a halogenated uridine for thymidine in DNA would sensitize to radiation is > 20 years old (14). The degree of sensitization depends on the percentage of DNA which is replaced by the thymidine analog. The bromenated and iodinated compounds have proved most efficacious. This sensitization occurs apparently equally in aerated and hypoxic cells at conventional dose rates. Little is known about the relative sensitization at low dose rates or with small radiation fractions. Differential radiosensitization in the case of these compounds depends on more rapid incorporation of the thymidine analog into the tumor cells as compared to surrounding normal tissues. This situation exists in sites such as CNS, lung, liver, and peripheral soft tissues.

A final and most interesting class of sensitizers for which a clinical rationale exists are those that modify or inhibit potentially lethal damage (PLD) repair. It has been shown in several systems, including plateau-phase cultured cells and tumors in vivo, that increased survival will occur under certain conditions after radiation exposure and before plating or other assay of cell survival (15,16). Most of the work to date has occurred in cultured cells and in a small number of tumors, and it is not known to what extent PLD occurs in normal tissues and whether or not compounds inhibiting it in tumors would also inhibit it to the same degree in normal tissues. Table 3 outlines a number of the mechanisms of action of PLD repair inhibition and a number of the drugs shown to be active either in cells in culture or in vivo in tumor systems. The best agents appear to be purine analogs which have been shown active both in vivo and in vitro (17,18). In addition, PLD repair is probably influenced by inhibitors of poly (ADP-ribose) synthesis, such as caffeine, and damage modifiers, such as dactinomycin. The major action, however, of most of the previously mentioned com-

TABLE 3.—PLD repair inhibitors

Mechanism of action	Active drugs	Systems evaluated
Alteration of intracellular nucleotide pool	3-Deoxyadenosine	Cultured cells,* tumor in vivo (x-ray)
	3-Deoxyguanosine	Cultured cells,* tumor in vivo (chemotherapy† + x-ray)
	2′,3′-Dideoxythymidine	Cultured cells*
	9-β-D-Arabinofuranosyladenine	Cultured cells,* tumor in vivo (chemotherapy†)
Inhibition of poly (ADP-ribose) synthesis	Caffeine	Cells in culture
	3 Amino-benzamide	Cells in culture
Modification of damage	Dactinomycin	Cells, in vivo systems
	Doxorubicin	Cells, in vivo systems

* SCC VII tumor cells in C3H/He.
†Bleomycin + cyclophosphamide.

pounds in the purine analog class occurs through alteration of the intracellular nucleotide pool, blockage of DNA synthesis, and thus repair of the radiation lesion. Much work is still needed to determine whether there will be differential blocking in tumor versus normal tissue and whether any of the compounds will prove to be sufficiently nontoxic in humans to act in this manner.

Hypotheses To Be Tested

Obviously, the first hypothesis which must be investigated and proven clinically is that hypoxic cells do exist in tumors and that they cause increased tumor resistance and a reduction in radiation control rates as a function of tumor type, stage, and site. Additional hypotheses include the efficacy of hypoxic cell chemical sensitizers in sensitizing hypoxic cells, if they do exist, and if they are important to a degree that can be clinically measured.

An additional hypothesis under active testing currently is that sufficient pyrimidine analogs can be incorporated into DNA to cause differential sensitization in clinical tumors such as malignant gliomas, liver metastases, and soft tissue sarcomas.

Hypotheses requiring testing for thiol depletors include (*a*) the degree to which they can enhance the effect of existing hypoxic cell sensitizers, and (*b*) the degree to which they will enhance tumor response on their own. These latter hypotheses cannot be tested without further preclinical animal data on the efficacy and toxicity of such compounds. Additional animal work is needed before the hypothesis that differential inhibition of PLD repair can occur in tumor versus normal tissue can be tested in the clinic.

CURRENT RESULTS OF CLINICAL TRIALS OF SENSITIZERS

Phase I Studies

Clinical trials to date have focused on nitroimidazole hypoxic cell sensitizers. Recently, five phase I clinical trials have been carried out with nitroimidazoles and one with a pyrimidine analog. These phase I evaluations of radiosensitizers are summarized in table 4. The dose-limiting toxic effects and the maximum tolerated doses (MTD) for a 4-6-week treatment course are shown in the table. Although 5-nitroimidazoles (eg, metronidazole) show a high MTD, their low sensitizing efficiency causes them to be inferior to the more efficient 2-nitro compounds. The drug development effort at the National Cancer Institute (NCI) has concentrated on lowering the lipophilicity of nitroimidazoles and restricting their access to the brain (19).

Two theories have been used to develop more efficient and less toxic nitroimidazole sensitizers. In the first approach, iv administration and shorter half-lives, as shown schematically in figure 1, have been employed. Since radiation is delivered almost instantaneously, there is no need for a prolonged half-life after its administration and maximal shortening of the half-life and reduction in the area under the curve will reduce toxicity. Also, iv administration allows higher peak tumor concentrations at the time of radiation delivery. The second approach has been the development of more hydrophilic nitroimidazoles by Brown and Workman (19) and Brown et al (20). Figure 2 demonstrates the relationship between the tumor-to-plasma and -brain ratios as a function of partition coefficient, and the improvements can be seen with desmethylmisonidazole (Ro-05-9963) and SR-2508. Compounds even more hydrophilic were shown to be less effective as sensitizers because of poor tumor penetration.

Table 5 compares misonidazole, desmethylmisonidazole, and the current results with SR-2508 in terms of the incidence of total peripheral neuropathy, peripheral neuropathy greater than grade 1, percent CNS effect, and percent ototoxicity as found in Radiation Therapy Oncology Group (RTOG) studies. From this table it can be seen that desmethylmisonidazole was superior to misonidazole, with a lower serious peripheral neuropathy rate and no CNS effects. Its MTD was, however, only slightly higher than that of misonidazole because of peripheral neuropathy. SR-2508 appears to be significantly better than the two previous compounds with a very low incidence of serious peripheral neuropathies and no instance to date of CNS injury or ototoxicity at doses as high as 30 g/m^2.

It should be emphasized that this phase I study is still under way and further data are expected. Table 6 compares the pharmacokinetics of the 2-nitroimidazoles tested in phase I. It can be seen that the iv preparations

TABLE 4.—Phase I clinical trials with radiosensitizers

Compound (type)	MTD	Limiting toxic effects
Metronidazole (5-nitro)	60 g/m^2	CNS, vomiting
Misonidazole (2-nitro)	12 g/m^2	CNS, PNS
Desmethylmisonidazole (2-nitro)	14 g/m^2	PNS
SR-2508 (2-nitro)	> 30 g/m^2	PNS
Ro-03-8799 (2-nitro)	In progress	CNS
BUdR (pyrimidine analog)	750 mg/m^2/12 hrs	Marrow, skin

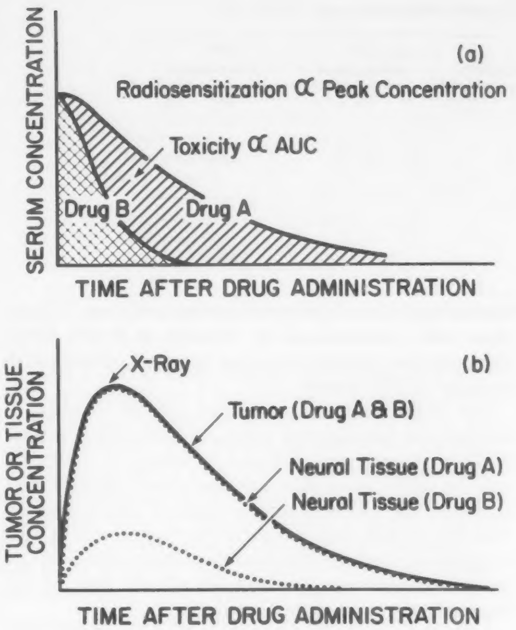

SERUM CONCENTRATION
(a)
Radiosensitization ∝ Peak Concentration
Toxicity ∝ AUC
Drug B
Drug A
TIME AFTER DRUG ADMINISTRATION
TUMOR OR TISSUE CONCENTRATION
X-Ray
(b)
Tumor (Drug A & B)
Neural Tissue (Drug A)
Neural Tissue (Drug B)
TIME AFTER DRUG ADMINISTRATION

FIGURE 1.—2 possible methods of reducing the neurotoxicity of a radiosensitizer while maintaining its efficacy are based on the fact that the radiosensitization depends on concentration of the sensitizer in the tumor at the time of irradiation, whereas the toxicity is determined by the total neural tissue exposure or drug concentration × time (area under the curve). Panel (a) shows that Drug B, which has a shorter serum half-life yet achieves the same peak serum concentration, should have less toxicity with equal efficacy. Panel (b) shows that a drug can be selected to be less toxic if it can be preferentially excluded from neural tissue. [This fig is modified from Brown and Lee, Pharmacokinetic Considerations in Radiosensitizer Development. *In* Radiation Sensitizers—Their Use in the Clinical Management of Cancer (Brady, ed). New York, Masson Publishing, 1980.]

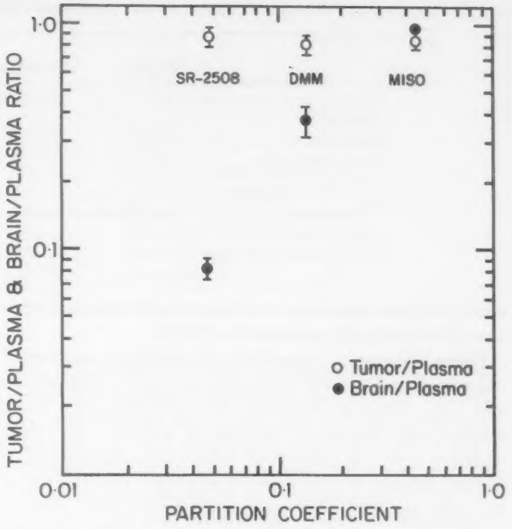


FIGURE 2.—Tumor-to-plasma and brain-to-plasma ratios of the 3 clinically evaluated radiosensitizers vs their partition coefficients. These data show that as the octinol:water partition coefficient decreases, there is maintenance of equal drug penetration into the tumor but a marked decrease of drug penetration into the brain and perhaps all neural tissues. DMM = desmethylmisonidazole and MISO = misonidazole. [These data are modified from Brown and Lee, Pharmacokinetic Considerations in Radiosensitizer Development. *In* Radiation Sensitizers—Their Use in the Clinical Management of Cancer (Brady, ed). New York, Masson Publishing, 1980. *See also* ref 19.]

all showed higher 1-hour blood levels at 1 g/m^2 than the oral preparation and that there was a gradual decrease in the beta-half-life as lipophilicity was decreased with a concomitant increase in the percentage of the parent compound recovered in the urine. Since it is thought that breakdown products make up the neurotoxic compounds, greater urine recovery of the primary compound is of great importance.

Currently, SR-2508 has only been tested in 3-week courses of nine doses, and it is unclear as to what will be the ultimate MTD, although it is possible that it could be as high as 40 g/m^2 delivered over 5 weeks. This dose level, which would allow 20 fractions of 2 g/m^2 coupled with the high tumor level achievable with an iv preparation,

TABLE 5.—Incidence of neuropathy at radiosensitizer doses > 9 g/m^{2*}

	N	% PNS	% PNS > grade 1	% CNS	% OTO	Dose (g/m^2)	
						Range	Mean
Misonidazole							
Phase I study	65	62	51	12	12	9–21	12
Phase II study	559	25	13	4	5		
Phase III study	967	14	4	3	1		
Desmethylmisonidazole							
Phase I study	90	47	16	0	4	9–18.9	11.6
SR-2508 (> 12 g/m^2)	29	24	7	0	0	21.6–31.5	22.4

*N = No. of patients, PNS = peripheral neuropathy, CNS = CNS effects, and OTO = ototoxicity.

TABLE 6.—Pharmacokinetic comparison of SR-2508, desmethylmisonidazole, and misonidazole[*]

	SR-2508	Desmethylmisonidazole	Iv misonidazole	Oral misonidazole
1-hr blood level (μg/ml)	45	41	48	30[†]
β-half-life (hrs)	5.4	6	9.3	13.8
% urine recovery in 24 hrs	71	62	23[‡]	30[‡]

[*] Normalized for a dose of 1.0 g/m^2. Values = mean.

[†] At 4-6 hrs.

[‡] ⅔ as desmethylmisonidazole.

could produce significantly greater tumor enhancement than that seen with misonidazole (21,22).

Phase I testing of halogenated pyrimidines was carried out with BUdR in 1962 by Bagshaw et al at Stanford (23) and with IUdR by Calabresi and colleagues (24). These studies were abandoned because it was felt the drugs were too toxic and caused too much enhanced response in normal tissues. In retrospect, it was clear that the radiation trial using BUdR was done at a site in which normal tissue enhancement of response of the oral mucosa was equal to tumor enhancement; thus, no therapeutic gain occurred. The IUdR study gave the compound up as a chemotherapeutic agent but did not investigate its activity as a radiosensitizer. More recent studies are discussed below.

Phase II and III Studies

Nitroimidazoles

Phase II studies have been carried out with the nitroimidazoles, with particular emphasis on these studies in the RTOG. The RTOG trials concentrated completely on misonidazole, but there was a randomized phase II study done with metronidazole by Uratsun et al (25) which demonstrated benefit in a small group of patients with malignant CNS tumors.

The RTOG carried out a large number of phase II studies with misonidazole which are summarized in table 7. These studies were designed to determine whether a specific combination of misonidazole and radiation fractionation was tolerated both in terms of drug and radiation toxicity and whether or not the response was at least as good as previous historical data. Suggestions of improved response were used to select specific sites for randomized controlled phase III trials (7).

More than one-half of the phase II studies listed were not carried on to phase III because of poor response or unacceptable radiation toxicity. The seven sites selected for controlled trials are listed in table 8. The patient numbers and interim results are updated as of the last analysis in June 1983. This table indicates the misonidizole dose employed, the size of the radiation fraction employed with the misonidazole dose, the number of patients randomized versus the number evaluable, and the tentative outcome. During the first 3 years of this series

TABLE 7.—Results of phase II RTOG misonidazole studies

Study site	Dose/fraction		No. of patients	Results compared to historical controls
	Misonidazole (g/m^2)	Radiation (Gy)		
Glioma	2.5	4 (1.5)	59	Equal
Glioma, recurrent	1.5	3	26	Equal-better
Glioma, neutrons	2.5	3fn	25	Equal-better
Brain metastases	2	6	40	Better
Head and neck, stage I	1.75	2.5, 2.1 (1.8)	50	Better
Head and neck, stage II	1	4	11	Equivocal
Lung	1.75	6	49	Better
Esophagus	1	4	43	Worse
Liver metastases	1.5	3	50	Equal
Sarcoma/melanoma	1.75	6	51	Equal
Bladder, stage I	1.5	4 (2)	11	Better/bowel toxicity
Cervix	1.25	4		Equivocal
Hemibody	4	6	39	Equal
Advanced pelvic	4	10	46	Equal
Bladder, stage II		2	19	Equal
Head and neck, stage III (random phase III)	1.5	4	42	Equal

TABLE 8.—Results of phase II RTOG misonidazole studies

Study site	Dose/fraction		Patients*	Preliminary results of closed studies
	Misonidazole (g/m^2)	Radiation (Gy)		
Glioma	2.5	4 (1.5)	318/269	Equal
Brain metastases	1/2	3/5	831/763	Preliminary-equal
Head and neck	1.75	2.5, 2.1 (1.8)	306/266	Trend to increased duration of local response/control
Lung, stage III	0.4	2	266/220	Equal
Lung, stage IV	1.75	6	117/96	No benefit
Liver metastases	1.5	3	202/185	Equal
Cervix	0.4	2	83/72	In progress

* No. randomized/No. evaluated at last analysis (June 1983).

of studies, 4-6-hour serum levels were required as well as a 24-hour serum level. The mean 4-6-hour serum levels obtained versus the dose in grams per square meter are shown in figure 3. With these large numbers of patients it can be seen that a dose of 2.5 g/m^2 was required to achieve between 80 and 90 $\mu g/ml$. With tumor concentrations of 50%-70% of this, the tumors were probably in the range of 45-60 $\mu g/ml$ at the maximal dose used in these trials. It can be seen, of course, that most of the trials were carried out at doses < 2.5 g/m^2.

Although initial predictions were that doses of 1 and 2 g/m^2 would yield enhancement ratios of 1.3-1.7, more recent animal data suggest that these predictions were too high (26-28).

Since tumor concentration is probably only 50%-70% of serum levels as discussed above, particularly with an orally administered compound, and since the sensitizer enhancement ratio is probably a linear function of concentration, it is likely that the maximal doses used in these trials gave sensitizer enhancement ratios in the range of 1.2-1.3. The misonidazole doses and the expected enhancement ratios based on more recent animal data from Brown (26) are shown in figure 4. It can be seen that the misonidazole clinical range at the maximal doses used could have probably achieved an enhancement ratio of 1.4 and probably less because of lower tumor concentration. In the same figure the expected clinical range achievable with SR 2508 is shown. An enhancement ratio

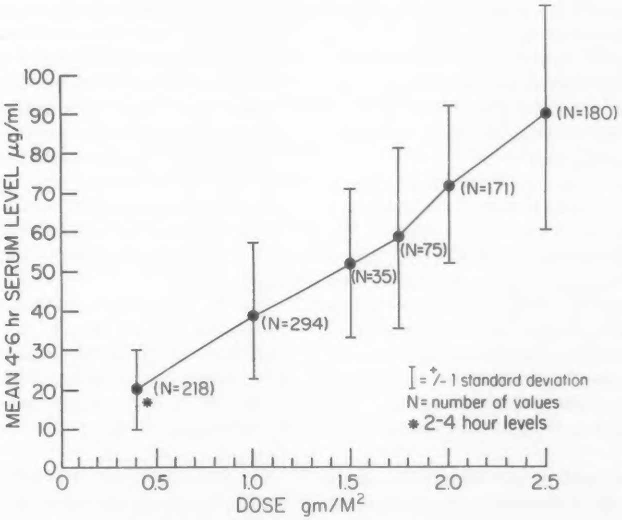


FIGURE 3.—Misonidazole serum levels from the 7 RTOG phase III studies. Mean 4-6-hr serum level ($\mu g/ml$) is given vs the oral misonidazole drug dose (g/m^2). These data show that there is a linear relationship between the oral dose of misonidazole and the serum level obtained.

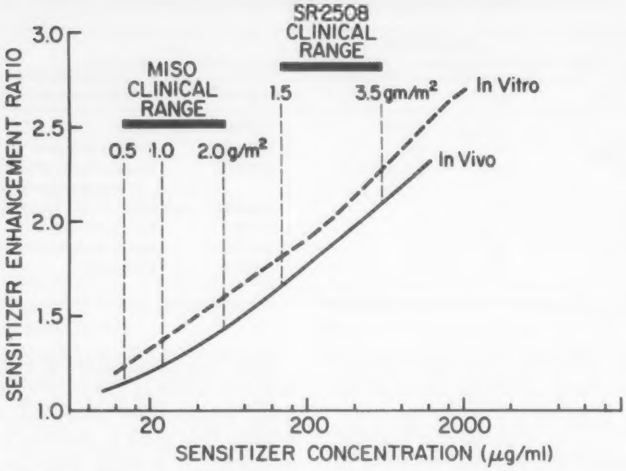


FIGURE 4.—Sensitizer enhancement ratio vs sensitizer concentration (μg/ml) is compared. Solid line data are a best fit from numerous in vivo experiments and dashed line data are from in vitro experiments. Clinical range of expected tumor levels from misonidazole by a single drug dose is given by the vertical dashed lines. The same is true for the clinical range of SR-2508. These data show that the sensitizer enhancement ratio anticipated from misonidazole is in the range of 1.1–1.3, whereas the sensitizer enhancement ratio anticipated from SR-2508 is in the range of 1.5–2.0.

> 2 can be expected, even with doses as low as 2 g/m^2, because of the high initial concentration achievable with iv administration of this compound.

The seven sites studied in phase III and shown in table 8 have so far shown essentially null results in preliminary analysis. The carcinoma of the cervix study is still in progress and one can make no statement as to efficacy. Two of the studies employed misonidazole with only some of the fractions, ie, those for glioma and head and neck cancer; those fraction sizes not delivered with misonidazole are shown in parentheses.

Since follow-up in the glioma study is longer than in the others and the survival time is short, it is fairly clear that these arms are equal and that results were null. The brain metastasis study requires further evaluation in terms of computed tomography response, which is now under way. There were no significant differences in survival seen among the four treatment arms which were delivered with two different misonidazole doses and two different radiation fraction sizes. However, survival in this disease may not be the major endpoint, and one must await the result of a histology by histology evaluation of tumor response.

The trial for head and neck cancer showed no significant difference in the initial complete response rate, although there is currently a trend toward a slightly higher duration of complete response in the misonidazole arm. The studies in lung cancer and liver metastases so far show no significant differences and it is probable that they will show a null result. All of these studies are in early analysis, however, and it will be several years before one can conclude that there was no benefit of misonidazole.

It can be seen from this table that some studies were carried out with very low doses of misonidazole given with each radiation fraction, a small number of radiation fractions with higher misonidazole doses, or in the two studies mentioned, with moderately high misonidazole doses with only a small proportion of the radiation fractions. At best, the misonidazole effect was diluted out or the dose was too low in most of these studies. In the few studies employing higher doses, a small number of fractions were involved and probably insufficient reoxygenation occurred for optimal response in conjunction with the sensitizer.

The sensitizer enhancement ratio achieved with misonidazole between 1.05 and 1.3 is probably not measurable clinically in terms of benefit when one considers that the tumors contain anywhere from 1% to 30% hypoxic cells. It is also likely that some of these tumor sites or histologies do not fail conventional radiotherapy because of hypoxic cells.

On the other hand, it is unlikely that all tumors lack important populations of hypoxic cells. It is necessary to test this hypothesis, however, with a sufficiently potent hypoxic cell sensitizer that would assure sensitization of the hypoxic cells with ratios between 1.5 and 2.0 with each radiation fraction so that the difference occurring, if any, could be detectable in clinical trials with reasonable numbers of patients.

The encouraging results to date in terms of toxicity of the phase I testing of SR-2508 coupled with its higher tumor concentration because of the iv administration and rapid tumor diffusion promise to allow enhancement ratios of at least 1.5 and perhaps 2.0, with up to at least 20 sensitizer administrations. This should allow for adequate testing of the hypothesis for the presence of hypoxic cells within tumors. Trials are planned for selected sites, however. It is necessary to use tumors which have a reasonable control rate with radiation alone, which have shown success with hyperbaric oxygen or transfusion or other evidence of hypoxic cells, and which have a major component of their failure at the local treatment site. Potential candidates include squamous cell carcinomas of the head and neck, cervix, and lung, and possibly adenocarcinoma of the prostate.

Thymidine Analogs

After the initial phase I evaluations and the small randomized phase II study of BUdR at Stanford, there was also a nonrandomized phase II study of BUdR carried out in Japan by Hoshino and Sano (29), which seemed to show a promising increase in survival for the high-grade gliomas treated in this study. The lack of controls and the mixture of lower-grade tumors made the results difficult to evaluate.

Recently, there has been a revival of interest in the use of BUdR in sites where the normal tissue uptake is low and where relatively rapid tumor growth occurs. A phase I study of iv rather than intra-arterial BUdR was carried out at the NCI (30), and it was found that there was reasonably good bioavailability by this route. It had been previously thought that intra-arterial administration was essential to achieve adequate concentrations. At this time it is not clear that particularly high concentrations are needed but rather prolonged exposure of the tumor cells so that high DNA incorporation can occur.

This phase I study has led to two phase II evaluations of BUdR as a sensitizer for malignant gliomas. One study at the NCI employs drug administration for 24 hours/day for 2 weeks, with rest periods and repeat administrations. A phase II study of malignant gliomas conducted by the Northern California Oncology Group employs 4-day 24-hour infusions and 3-day rest periods over a 5–6-week course. Both studies use conventional radiation with approximately 4500 rads (whole-brain) and a boost to the local lesion to 6000 rads.

Currently, toxic effects have included a small number of idiopathic severe skin eruptions and a rather large number of skin eruptions in sites exposed to white light. Significant bone marrow toxicity has also occurred, with platelet suppression being most marked and requiring dose limitations to about 700 mg/m^2/day with either of the two schedules mentioned. It is too early to evaluate the effects of this treatment on survival of these patients or to allow direct comparison with historical controls until larger groups are accumulated with longer follow-up. Further work on routes of administration, minimization of toxicity, and potential use of IUdR is now under way at the NCI.

The potential in the clinic for thiol depletors and blockers of PLD repair is yet to be realized. Further animal studies are needed to prove that combinations of thiol depletors and sensitizers can be administered without severe toxicity before phase I clinical testing can begin. Much additional evidence is needed that there would be a differential effect of PLD repair blockers between animal tumor and normal tissue studies before clinical trials of these compounds can be contemplated.

RADIOPROTECTORS

During the initial radiochemical events the presence of sulfhydryl compounds can reduce the permanent lesions in DNA by up to a factor of 3. It is highly likely that endogenous sulfhydryls cause natural radioprotection and that the addition of exogenous sulfhydryls can cause further protection through repair of free radicals in DNA as well as neutralization of free radicals formed in cellular water. Protection against ionizing radiation by sulfhydryl-containing compounds was first described by Patt et al in the late 1940s (31). Since radioprotection had potential military applications, an extensive synthesis program was undertaken by the US Army in an attempt to find effective orally administered compounds. From this program came a number of promising structures, many of which were more effective as protectors than the original compound. Some of the most potent compounds were made less toxic by covering the sulfhydryl group with a phosphate group.

Yuhas and Storer (5) discovered in 1969 that one of these compounds, WR-2721, showed differential chemoprotection of normal versus malignant tissues.

Previously, cysteamine and other compounds had been proven to cause essentially equal protection to tumor and normal tissue in studies of protection both against radiation and against cytotoxic chemotherapy.

Several potential reasons for this differential protection were put forward. Harris and Phillips (32) proposed that the major reason for the differential was a decreased efficacy of sulfhydryl compounds in the protection of hypoxic cells. In addition, a differential distribution of the compound with preference toward higher concentrations in normal tissues early after administration was shown in pharmacologic studies (33). More recently, Yuhas (34) has shown differential absorption kinetics between normal tissues and tumors, probably based on the very low lipophilicity of WR-2721 (35).

Based on these factors it can be well-supported that in many tumors greater protection occurs in comparable normal tissues than in the tumor. This is particularly evident at high doses per fraction where the lack of protection of hypoxic cells is dominant but can also be seen in many tumors at lower doses per fraction. This is by no means universal, however, and a number of studies have shown fairly high degrees of protection of certain tumors, particularly at low doses per fraction. An overview of all animal data available, however, leads one to conclude that there is probably significant differential protection of tumor over normal tissue, with certain tumor types and certain normal tissues showing high degrees of protection.

Table 9 shows some of the dose-modifying factors available for critical (ie, life-threatening) normal tissues undergoing radiotherapy. Obviously, there are a number of tissues for which this information is missing, but it is clear from the information so far available that high degrees of protection can be seen in bone marrow and small intestine (acute effects) and liver (late effects). Protection has been rather disappointing in lung and in kidney.

In addition, for certain acute reactions in non-life-threatening tissues such as oral mucosa, parotid glands, and skin, dose-modifying factors in the range of 2 have been found. Only rare tumors have shown protection to this degree.

Thus, one can expect reasonable protection of the oral mucosa and salivary glands, the skin, the small intestine, and the liver. These animal data have significant value in predicting which site should be used for initial phase II clinical trials of a successful radioprotector.

Hypotheses To Be Tested

The first hypothesis that must be evaluated is that radioprotectors can be given in sufficient doses to humans to cause significant radioprotection in a reasonable number of normal tissues. The second hypothesis is that differential concentration will occur in human normal tissues as compared to tumors and likewise differential protection. The third hypothesis is that higher total radiation doses will be deliverable to humans given WR-2721 and that these higher radiation doses will yield higher control rates because of differential protection.

TABLE 9.—WR-2721 dose-modifying factors for critical type 1 normal tissues

Tissue	Dose-modifying factors	
	Acute	Chronic
Brain	1.0	—
Spinal cord	—	—
Lung	—	1.2
Heart	—	—
Stomach	—	—
Small intestine	1.8-2.1	—
Large bowel	—	—
Liver (chromosomes)	—	2.7
Kidney	—	1.5
Bone marrow LD30	2.2-2.7	—
Bone marrow colony-forming units	3.0	—

All of these hypotheses must be evaluated before the future potential for radioprotectors in clinical therapy is known.

Clinical Trials

The first evaluation of WR-2721 using the iv compound was carried out in Japan by Tanaka and Sugahara (36).

They delivered the protector WR-2721, which was manufactured in Japan, with daily administrations during head and neck radiotherapy and found that the maximum acceptable dose was approximately 100 mg/m^2/day. Nausea was the main limiting factor; no other significant toxic effects were noted.

Phase I testing of the drug in the United States was begun by Kligerman at the University of New Mexico and was continued at the University of Pennsylvania with an escalating single-dose study (37). Initially, the drug was given with a prolonged infusion time because of nausea and vomiting seen early in the trial. More recently, it has been found that rapid infusions produce no more nausea and are more likely to produce differential protection. Doses as high as 1300 mg/m^2 have been given with slow infusions without serious toxicity and as high as 740 mg/m^2 with rapid (10-minute) infusions. This dose is now considered the current MTD or recommended dose, although attempts to increase it further are still under way by the group at the University of Pennsylvania.

A phase I clinical trial is being carried out by the RTOG at several of its member institutions which have also been active in the phase I sensitizer testing. This study reached a dose of 170 mg/m^2 four times per week and encountered several patients with idiotypic reactions of fever and hypotension. The study was interrupted to determine that this was not due to impurities in the drug or pyrogens and the study was then resumed. Currently, doses of up to 300 mg/m^2 delivered four times per week have been achieved. In the near future, this schedule will be extended for a 6-week period and then further escalation will be attempted.

It is now recognized that hypotension occurs in about 20% of patients and is not dose-related. The idiosyncratic reactions with fever and allergic symptoms are rare and again not dose-related. Nausea and vomiting occur with most administrations and will probably be the limiting factor, with multiple administrations over 3 and 6 weeks.

The RTOG has started a phase I-II study evaluating bone marrow protection in patients receiving hemibody radiation with doses of 600 rads plus initially 450 and now 740 mg/m^2 of WR-2721. Although only a small number of patients have been entered in the trial, preliminary results suggest decreased hematologic side effects as compared to patients treated without WR-2721.

Future Plans for Radioprotectors

A pharmacologic assay for WR-2721 for use in humans is urgently needed. Although radiolabeled compounds may be used, it is not certain with these assays whether one is measuring the parent compound, the diphosphorylated form, or disulfides. Concentrated efforts have gone on at several institutions to develop high-performance liquid chromatographic-type assays in combination with complex chemical separations to assay both the parent compound and its diphosphorylated form. These assays are nearing completion and preliminary curves have already been obtained in humans at the University of Pennsylvania. They suggest a rapid distribution phase and then a long half-life of what is probably serum-bound compound. To date, no biopsies of human normal tissues or tumors have been obtained to determine the concentration in these sites. This information is essential before further trials can be planned.

If the pharmacologic data indicate good concentration in normal tissues and low concentration in tumor, then phase II studies can be developed. The first study will probably be one employing multiple skin nodules metastatic from breast melanoma and other sites which will be given graded radiation doses with and without WR-2721; both skin reactions and tumor responses will be evaluated to determine whether skin protection and/or tumor protection occurs and whether there is a differential effect. If this study is positive, a phase II study would be mounted in the head and neck area and perhaps in the pelvis, where radiation doses will be escalated looking for protection against injury to the mucous membranes, salivary glands, and skin in the head and neck region as well as the subcutaneous tissues and for protection against bowel injury in the pelvis. Any plans for phase III studies would depend on the outcome of these. If escalation to higher doses with at least equal tumor response can be achieved, then phase III trials would be indicated.

Additional methods exist for the protection of certain normal tissues. They include the insertion of balloon catheters or the local administration of vasoconstrictors or starch microspheres to reduce vascular profusion and therefore oxygen tension in critical organs such as the kidney included in the radiation field. These approaches have had fairly extensive large-animal testing and, in the case of balloon catheters and vasoconstrictors, very limited phase I human evaluations. They may receive more attention in the future.

CONCLUSION

Chemical radiation modification has reached a high level of clinical investigation. It rests on a firm biologic basis, ie, the potential for modifying the initial radiochemical lesions and therefore either augmenting the number of these lesions in the tumor cell or reducing the number of lesions in the normal tissue.

Although initial clinical trials with misonidazole were disappointing, it is clear that this drug was inadequate because of its neurotoxicity and did not achieve high enough sensitizer enhancement ratios. Adequate evidence remains for the presence of hypoxic cells in some human tumors and the newest nitroimidazole, SR-2508, promises to be sufficiently nontoxic to be deliverable at high doses with up to 20 radiation fractions. Further enhancement of sensitization may occur through thiol depletion as well as the addition of compounds with different toxicity to achieve the ultimate goal of sensitization at the maximal level, ie, 3.

Radioprotection depends on differential concentration and protection in tumor. WR-2721 is progressing slowly but definitely in clinical trials toward phase II and perhaps phase III evaluation in humans as pharmacologic assays develop and the MTD are established.

REFERENCES

1. GRAY LH, CONGER AD, EBERT M, ET AL. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 26:638-648, 1953.
2. HENK JM, and SMITH CW. Radiotherapy and hyperbaric oxygen in head and neck cancer. Lancet 2:104-105, 1977.
3. WATSON ER, HALNAN KE, DISCHE S, ET AL. Hyperbaric oxygen and radiotherapy: a medical research council trial in carcinoma of the cervix. Br J Radiol 51:879-887, 1978.
4. ADAMS GE, and DEWEL DL. Hydrated electrons and radiobiological sensitization. Biochem Biophys Res Commun 12:473-477, 1963.
5. YUHAS JM, and STORER JB. Differential chemoprotection of normal and malignant tissues. JNCI 42:331-335, 1969.
6. THOMLINSON RH, and GRAY LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer 9:539-549, 1955.
7. PHILLIPS TL, WASSERMAN TH, STETZ J, ET AL. Clinical trials of hypoxic cell sensitizers. Int J Radiat Oncol Biol Phys 8:327-334, 1982.
8. STRATFORD IJ. Mechanisms of hypoxic cell radiosensitization and the development of new sensitizers. Int J Radiat Oncol Biol Phys 8:391-398, 1982.
9. SLOVITER HA, and PETKOVIC M. Dispersed fluorochemicals as substitutes for erythrocytes in intact animals. J Appl Physiol 27:666-669, 1969.
10. TREMPER KK, FRIEDMAN AE, LEVINE EM, ET AL. The pre-operative treatment of severely anemic patients with a perfluorochemical oxygen-transport fluid, Fluosol-DA. N Engl J Med 307:277-283, 1982.
11. MOORE BA, PALCIC B, and SKARSGARD LD. Radiosensitizing and toxic effects of the 2-nitroimidazole Ro-07-0582 in hypoxic mammalian cells. Radiat Res 67:459-473, 1976.
12. BUMP EA, YU NY, and BROWN JM. Radiosensitization of hypoxic tumor cells by depletion of intracellular glutathione. Science 217:544-545, 1982.
13. HODGKISS RJ, and MIDDLETON RW. Enhancement of misonidazole radiosensitization by an inhibitor of glutathione biosynthesis. Int J Radiat Biol, in press.
14. DJORDJEVIC B, and SZYBALSKI W. Genetics of human cell lines. III. Incorporation of 5-bromo and 5-iododeoxyuridine into the deoxyribonucleic acid of human cells and its effects on radiation sensitivity. J Exp Med 112:509-531, 1960.

15. PHILLIPS RA, and TOMLACH LJ. Repair of potentially lethal damage in x-irradiated HeLa cells. Radiat Res 29:414-432, 1966.
16. LITTLE JB, HAHN GM, FRINDEL E, ET AL. Repair of potentially lethal damage in vitro and in vivo. Radiology 106:689-694, 1973.
17. NAKATSUGAWA S, SUGIHARA T, and KUMAR A. Purine nucleoside analogues inhibit the repair of radiation-induced potentially lethal damage in mammalian cells in culture. Int J Radiat Biol 41:343-346, 1982.
18. NAKAGSUGAWA S, KUMAR A, ONO K, ET AL. Increased tumor curability by radiotherapy combined with PLDR inhibitors in murine cancer. *In* Prospective Methods of Radiation Therapy in Developing Countries. Proceedings of International Atomic Energy Agency Seminar, Kyoto, Japan, Aug 31-Sept 4, 1981, pp 77-86.
19. BROWN JM, and WORKMAN P. Partition coefficient as a guide to the development of radiosensitizers which are less toxic than misonidazole. Radiat Res 82:171-190, 1980.
20. BROWN DM, YU NY, BROWN JM, ET AL. In vitro and in vivo radiosensitization by 2-nitroimidazoles more electron-affinic than misonidazole. Int J Radiat Oncol Biol Phys 8:435-438, 1982.
21. COLEMAN CN, WASSERMAN TH, PHILLIPS TL, ET AL. Initial pharmacology and toxicology of intravenous desmethylmisonidazole. Int J Radiat Oncol Biol Phys 8:371-375, 1982.
22. COLEMAN CN, WASSERMAN TH, URATSUN RC, ET AL. Preliminary results of the phase I trial of the hypoxic cell sensitizer SR2058. Int J Radiat Oncol Biol Phys 9(Suppl 1):96, 1983.
23. BAGSHAW MA, DOGGETT RLS, SMITH LC, ET AL. Intra-arterial 5-bromodeoxyuridine and x-ray therapy. Radiology 99:886-894, 1967.
24. CALABRESI P, CREASEY WA, PRUSOFF WH, ET AL. Clinical and pharmacological studies with 5-iodo 2′-deoxycytidine. Cancer Res 23:583-592, 1963.
25. URATSUN R, BAND P, CHAPMAN JD, ET AL. Radiation and high-dose metronidazole in supratentorial glioblastomas. N Engl J Med 294:1364-1367, 1976.
26. BROWN JM. Clinical trials of radiosensitizers: what should we expect? Int J Radiat Oncol Biol Phys, in press.
27. DENEKAMP J, and STEWART FA. Sensitization of mouse tumours using fractionated x-irradiation. Br J Cancer 37(Suppl III):259-263, 1978.
28. HILL RP, and BUSH RS. The effect of misonidazole in combination with radiation dose fractionation. Br J Cancer 37(Suppl III):255-258, 1978.
29. HOSHINO T, and SANO K. Radiosensitization of malignant brain tumors with bromouridine (thymidine analogue). Acta Radiol Ther Phys Biol 8:15-26, 1969.
30. KINSELLA TJ, RUSSO A, and MITCHELL JB. A phase I study of intermittent intravenous bromodeoxyuridine (BUdR) and conventional brain irradiation. Int J Radiat Oncol Biol Phys, in press.
31. PATT HM, TYREE EB, STRAUBE RL, ET AL. Cysteine protection against x-irradiation. Science 110:213-214, 1949.
32. HARRIS JW, and PHILLIPS TL. Radiobiological and biochemical studies of thiophosphate compounds related to cysteamine. Radiat Res 46:362-379, 1971.
33. WASHBURN LC, HAYES RL, CARLTON J, ET AL. Distribution of WR-2721 in normal and malignant tissues of mice and rats: dependence on drug dose, tumor type and species. Radiat Res 59:475-484, 1974.
34. YUHAS JM. Active versus passive absorption kinetics as the basis for selective protection of normal tissues by WR-2721. Cancer Res 40:1519-1524, 1980.
35. YUHAS JM, DAVIS ME, GLOVER D, ET AL. Circumvention of the tumor membrane barrier to WR-2721 absorption by reduction of drug hydrophilicity. Int J Radiat Oncol Biol Phys 8:519-522, 1982.
36. TANAKA Y, and SUGAHARA T. Clinical experiences of chemical radiation protection in tumor radiotherapy in Japan. *In* Radiation Sensitizers (Brady L, ed). New York, Masson Publishers, 1979, pp 421-425.
37. BLUMBERG A, NELSON DF, GRANKOWSKI M, ET AL. Clinical trials of WR-2721 with radiation therapy. Int J Radiat Oncol Biol Phys 8:561-564, 1982.

Particle-Beam Radiation Therapy 1983: Evaluation and Recommendations[1]

Melvyn P. Richter and Morton M. Kligerman[2,*]

Comprehensive summaries have recently appeared recording developments in particle-beam research in biology, physics, and medicine (1–3). Selected American and foreign centers have been using neutrons primarily and protons, pions, and heavy ions less commonly in clinical trials, but the therapeutic efficacy of these high-technology beams is unclear. The following issues need to be resolved: (*a*) Can specific particles alone or combined with surgery and chemotherapy produce local-regional control in selected clinical problems greater than that seen with state-of-the-art x-ray therapy? (*b*) Can particles significantly enhance disease- and complication-free survival more than x-rays to justify their greater cost? This paper briefly reviews the reasons accounting for the uncertainty in this area of radiation oncology and will propose means to clarify the status of particle therapy over the next 5 years as new hospital-based neutron facilities join the existing US particle program (4) (table 1).

BACKGROUND

Standard external-beam radiotherapy uses either electromagnetic waves (photons) or electrons (beta rays). High-energy photons are produced by either electronic devices (linear accelerators, betatrons) or radioactive sources (cobalt-60). External-beam electrons are generated by electronic devices only. The x, gamma, or beta rays produce a characteristic low-density ionization pattern in tissue as compared to the higher density ionizations produced by particles such as neutrons, pions, and heavy ions. Linear energy transfer (LET), a measure of ionization density, is the amount of energy released per micron of matter along the path of any ionizing particle. Greater than fifty kiloelectronvolts deposited per micron of tissue is considered high LET. The LET of an ionizing particle is directly related to that particle's charge (Q) and velocity (V) by the formula Q^2/V.

Uncharged x and gamma rays and neutrons (uncharged nuclear particles) deposit energy exponentially as they pass through tissue. Charged particles such as protons, pions, and heavy ions have a discrete range in tissue which is relative to the energy of the particles. The maximum amount of ionizing energy is deposited at the end of the particle range, the Bragg peak, and can be shaped to the tumor volume. Low-LET x-rays produce ionizations through interactions with orbital electrons. Neutrons, however, interact principally with the atomic nucleus by proton recoil.

For a given dose in rad, high-LET beams produce more cytotoxic radiation events than low-LET beams. In contrast, the relatively sparse ionizations of low-LET beams may allow critical molecules such as DNA to escape nonrepairable radiation damage (5). Additionally, the degree of cellular radiation damage is directly dependent on tissue oxygen concentration: the oxygen effect (6). Hypoxia is dose-modifying for low-LET radiation and less so for high-LET beams (7). With low-LET beams, therefore, hypoxia favors tumor protection since practically all tumors contain some percentage of hypoxic cells.

The ability to repair sublethal and potentially lethal radiation damage is also related to the LET of the beam (8). For high-LET beams, such repair capacity is reduced for both tumor and normal tissue. However, the relative reduction in repair ability in tumor versus normal tissue is not clear, so that the effect of this characteristic on the therapeutic ratio is still to be determined. The cell lethality produced by low-LET radiation is highly dependent on the cell-cycle phase. The relative radiosensitivity of most cell types to low-LET beams is maximal in M and G_2 phases. The sensitivity of cells to high-LET beams is less cell-cycle dependent. This property, in addition to the decreased capacity of cells to repair high-LET radiation damage, makes fractionation less significant with high-LET radiation. These radiobiologic factors together with the reduced oxygen effect result in an increased relative biologic effectiveness (RBE) of high-LET radiation as compared to conventional radiation and are the basis for the research interest in high-LET radiotherapy.

All qualities of radiation can produce deleterious late effects which may obviate any therapeutic gain obtained

---

[1] Supported in part by contract N01-CM-97314 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[2] Department of Radiation Therapy, University of Pennsylvania and the Fox Chase Cancer Center, Philadelphia.

[*] *Reprint requests to:* Morton M. Kligerman, MD, Department of Radiation Therapy, Hospital of the University of Pennsylvania, 3400 Spruce St, Philadelphia, PA 19104.

TABLE 1.—Characteristics of current particle-beam therapy facilities in the US*

Location	Equipment characteristics†	Status
Neutron Therapy Facilities		
Cleveland Clinic Foundation-Lewis Research Center (NASA), Cleveland, OH (45 mins from Cleveland Clinic)	Physics cyclotron; horizontal- and vertical-treatment beams; 42 MeVp-Be or 25 MeVp-Be	Operational
Fermi National Accelerator Laboratory (Fermilab), Batavia, IL (30 miles from downtown Chicago)	Physics machine (injector for synchrotron); horizontal-treatment beam; 66 MeVd-Be	Operational
Fox Chase Cancer Center-Hospital of the University of Pennsylvania (FCCC-HUP), Philadelphia (in FCCC)	d-T generator designed for clinical use; isocentric-treatment capability; 14-Mev neutrons from d-T reaction	Operational
M. D. Anderson Hospital and Tumor Institute (MDAH), University of Texas, Houston (in MDAH)	Cyclotron therapy system designed for clinical use; 1 isocentric-treatment beam and 1 horizontal beam; 42 MeVp-Be	Operational
University of California at Los Angeles (UCLA) (to be Wadsworth VA Medical Center)	Cyclotron therapy system designed for clinical use; 1 isocentric-treatment beam and 1 horizontal beam; 42 MeVp-Be	Under construction
University of Chicago, IL (in University Hospital)	Cyclotron therapy system with 1 horizontal beam; 8-Mev d-> d	Operational
University of Washington, Seattle (in University Hospital)	Cyclotron therapy system designed for clinical use; 1 isocentric-treatment beam and 1 horizontal beam; 48 MeVp-Be (variable energy 32-48 Mev)	Early 1984
Proton Therapy Facility Massachusetts General Hospital (MGH)-Harvard University Cyclotron, Cambridge (20 mins from MGH)	Physics cyclotron; 160-Mev protons; approximate range in water of 15.9 cm; horizontal-treatment beams	Operational
Helium and Heavy Ion Therapy Facility University of California, Berkeley, Lawrence Laboratory (12 miles from UCSF Hospital)	Physics synchrocyclotron; approximate range in water of 26 cm; 930-Mev helium ions; horizontal-treatment beam	Helium ions operational
	Physics machine (combination of a heavy ion linear accelerator and a synchrotron); high-energy beams with approximate ranges in water of ^{12}C-26 cm, ^{20}Ne-24 cm, ^{28}Si-20 cm, ^{40}Ar-16 cm; 2 horizontal-treatment beams	Heavy ions operational

*Modified from Pistenmaa DA, and Mahoney FJ. Int J Radiat Oncol Biol Phys 8:2177-2180, 1982.

†Mev = million electron volts; p-Be = protons bombarding Berylium; d-Be = deuterons bombarding Berylium; d-T = deuterons bombarding tritium; and d-> d = deuterons bombarding deuterium.

from improvement in local tumor control. The vascular-connective tissues, which generally are more slowly proliferating than tumor, express late radiation injury as fibrosis, ulceration, necrosis, and loss of function. Hydrogen-dense tissues also absorb a higher dose relative to muscle equivalent tissue when irradiated with neutrons. While the neutron RBE for late effects on muscle is approximately 3, that for brain and spinal cord may be as high as 5-7. This increased RBE in fat-rich tissue is not fully explained by differential dose absorption.

The RBE for high-LET beams has been shown to increase with treatment schedules using smaller dose increments protracted over longer time periods. This suggests that to reduce severe late effects, the total dose should be given in the shortest time with the least number of fractions compatible with acute tissue tolerance (9).

Clinical neutron irradiation was initiated by Stone and his colleagues in 1938 at the University of California, Berkeley, using a cyclotron adapted for radiation treatment. It produced fast neutrons equivalent in depth-dose characteristics to 250-kvp x-rays. Several hundred patients with diverse advanced tumors were treated, some of whom had persistent or recurrent disease following orthovoltage x-ray treatment or surgery. This trial was discontinued in 1942 due to the war effort. A review by Stone (10) conducted in 1948 led him to conclude that "Neutron therapy . . . resulted in such bad late sequelae in proportion to the few good results that it should not be continued." His warning caused the suspension of the American clinical particle program until 1972 when the MDAH, utilizing the Texas A & M variable energy cyclotron (TAMVEC), began phase I pilot studies supported by the National Cancer Institute (NCI). Within 1½ years, phase I trials were also begun at the University of Washington, Seattle, and the Naval Research Laboratory, Washington, DC, which was staffed by members of the George Washington University Department of Radiation Therapy. The renewed American interest in neutron radiotherapy followed the encouraging results in neutron radiation biology conducted in London at the Hammersmith Hospital utilizing the first cyclotron dedicated to biomedical research. The Hammersmith clinical neutron trials began in 1966 supported by the Medical Research Council.

As part of the British preclinical investigations, Bewley et al (11) demonstrated that the RBE for neutrons compared with x-rays was significantly different when neutrons were administered in single as contrasted with multiple fractions. The RBE was 30% greater with multiple fractions. Stone had determined his tumor doses on single-dose experiments, but he treated most of his patients with multiple fractions. Unaware of the increased neutron RBE with fractionation, he had inadvertently overdosed his patients, delivering a mean x-ray equivalent dose of 7000 ± 500 rad, with some patients receiving doses as high as 11,000 rad (12). A high-dose effect, not necessarily an intrinsic neutron effect, accounted for the severe late toxicity that Stone observed.

EQUIPMENT

Neutron-beam therapy has been delivered principally on physics research machines adapted for medical practice. In comparison with modern supervoltage x-ray equipment, these machines have been deficient in either their depth-dose or beam-direction properties. Cyclotrons at the Hammersmith Hospital, Western General Hospital (Edinburgh), and the University of Washington, for example, have produced neutron beams with depth-dose characteristics comparable to 250–660 kvp, inadequate for the curative radiotherapy of deep-seated lesions. All of the original US and foreign units except Edinburgh have been limited to fixed horizontal beams necessitating treatment of patients in a sitting or standing position, particularly suboptimal when delivering radiation to abdominal and pelvic neoplasms where large volumes of gravity-dependent small bowel fall into the treatment volume. Also, these units have permitted only limited shaping of the beam to the tumor volume so that a large amount of normal tissue has been included in the neutron treatment. Some units, such as TAMVEC, were distant from the primary cancer center. This has interfered with patient and physician accessibility. Furthermore, treatment delays have hampered the particle trials since medical research has competed for beam-time with basic physics research. For these reasons, the Committee for Radiation Oncology Studies recommended to the Radiation Therapy Development Branch, NCI, that hospital-based neutron facilities be established, housing equipment designed according to current minimum standards for external-beam radiation delivery. Three of the four facilities are to be operational by late 1983 to early 1984: FCCC-HUP, MDAH, and the University of Washington Hospital, Seattle. The UCLA unit is presently under construction at the Wadsworth VA Medical Center. The FCCC-HUP program will utilize deuterons bombarding a tritium target (d-> T generator) producing 14-mev monoenergetic neutrons. The three other units are high-energy cyclotrons producing neutrons by bombardment of protons on beryllium, all of which have similar energy spectra. All four machines are isocentrically mounted, allowing for the patient to be immobilized in an optimal treatment posture while multiportal therapy is directed to the target volume of interest. All machines have neutron depth-dose profiles at least equivalent to cobalt-60 (FCCC-HUP) or to 6-mev linear accelerators (MDAH, University of Washington, and UCLA). Existing non-hospital-based units at the Cleveland Clinic Foundation-Lewis Research Center (NASA) and the Fermi National Accelerator Laboratory in Batavia, IL, will continue to conduct neutron clinical trials. A non-NCI-supported hospital cyclotron is present at the University of Chicago producing neutrons similar in penetration to cobalt-60. Heavy ion research continues at the University of California, Berkeley, Lawrence Laboratory, and proton research continues at the MGH-Harvard University Cyclotron (table 1).

PAST TREATMENT PROGRAMS

In the conduct of the neutron particle programs, there has been marked variation among institutions in fractionation schedules, tumor doses, techniques of treatment delivery, and the variations resulting from treatment with either neutrons alone or neutrons combined with x-rays, the "mixed beam," which makes intercomparison of data difficult. Centers such as Hammersmith and Edinburgh report their doses as neutrons alone, while Amsterdam and the Fermilab report a total dose including both the primary neutron dose and the lesser gamma component, which all neutron generators produce. In the US, some institutions have utilized "equivalent dose," a composite of the x-ray dose and the particle dose, modified by an experimentally derived RBE. The Hammersmith dose is 1560-rad neutron in 12 fractions over 4 weeks; Edinburgh, using a similar beam, prescribes the same total neutron dose in the same overall elapsed time but gives 20 daily fractions instead of 12. Amsterdam chose the same number of fractions and elapsed time as Edinburgh but adopted higher (10%–30%) treatment doses. This was based on preclinical and clinical RBE estimates made with their 14-Mev monoenergetic d-> T neutron beam. Hammersmith and Edinburgh have tended to treat limited target volumes encompassing principally gross disease and omitting prophylactic irradiation to potential sites of metastatic spread. The American groups, however, have used more extensive treatment volumes to encompass potential lymph node drainage, consistent with standard radiation-therapy practice in the US (13). While European centers have emphasized pure neutron-beam programs, all American investigators, except the Fermilab, have pursued mixed-beam research. Generally, mixed-beam treatment has consisted of two treatments with neutrons on Tuesdays and Thursdays combined with three treatments with x-rays on the other weekdays. The decision to adopt mixed-beam therapy as the standard neutron regimen was arrived at early in the course of the US clinical trials. At MDAH, for example, phase I-II trials on gynecologic tumors were begun in April 1973 and the mixed-beam schedule was introduced 16 months later. A total of 17 patients were treated by neutrons alone, 11 of whom had advanced carcinoma of the cervix; 20 patients were treated by neutron boost, 17 of whom had advanced cervical cancer refractory to initial photon therapy (14), before pure neutron treatment was abandoned in favor of mixed-beam. For locally advanced carcinoma of the head and neck, the MDAH adopted mixed-beam in September 1974 as its neutron arm after 49 patients completed neutron-only therapy (15). In both instances, toxicity was felt to be less with mixed-beam programs.

Despite the above limitations, a number of reports support further neutron study. The Medical Research Council phase III advanced head and neck trial conducted at the Hammersmith Hospital demonstrated a 75% local control rate in the neutron-treated group as compared with 19% local control in the photon arm at 2 years (16). This study included patients with stage III-IV squamous cell carcinoma and adenocarcinoma of salivary gland origin. In a similar study of patients with advanced head and neck cancer, the Amsterdam group reported a local control rate of 44% with neutrons alone (17). In RTOG Protocol 76-10, a phase III trial comparing mixed-beam with conventional x-irradiation in patients with advanced inoperable squamous cell carcinoma of the head and neck, the records of 326 patients show no trend in improved local-regional control at the primary site or overall disease-free survival. In that study, however, there appears to be an advantage to the mixed-beam arm in control of cervical adenopathy at 2 years (18). Patients with salivary gland tumors treated with neutrons alone have shown control rates ranging from 82% (33 of 40 patients) at Hammersmith and 72% (18 of 25) at Amsterdam to 57% (nine of 16) at Edinburgh (19). For comparison, in 104 patients with malignant salivary gland tumors treated by x-irradiation with surgical salvage, Rafla-Demetrious (20) reports that 43% were controlled locally for 1-10 years. At MDAH, ten of 15 patients with locally advanced sarcomas treated with neutrons alone were locally controlled. Of six patients given a neutron boost to gross disease following 3500-5000 rad of conventional x-ray therapy, five were locally controlled without significant toxicity (21). In RTOG Protocol 77-05, a phase II study of neutron radiotherapy in the treatment of patients with either high-grade or advanced-stage carcinoma of the bladder, 11 of 14 patients treated with mixed-beam preoperatively showed no evidence of viable tumor in the cystectomy specimen (22). With conventional preoperative radiotherapy, 16% (23)-33% (24) of cystectomy specimens can be expected to be tumor-free.

RECOMMENDATIONS

Every effort should be made to reach a decision about the value of particle therapy by establishing clear-cut goals that can be accomplished in the shortest possible time through properly designed studies. To this end, the following guidelines for all particle programs in the US are offered:

1. Identify a limited number of optimal test sites to be studied serially by all particle users. Such sites should have a high local-regional recurrence rate in some stages following present customary therapy. Squamous cell carcinoma of the head and neck (stages III and IV) and posterior third of tongue (stage II) should be the first choice for study. Dr. Catterall at Hammersmith has demonstrated her greatest success here. Her data should be corroborated, using her dose-fraction schedule, free of the constraints attributed to limitations in early neutron machine technology. Second, patients with transitional cell carcinoma of the bladder (stage B-1, grade III, and stages B2-C of all histologic grades), those with inoperable or recurrent adenocarcinoma of the rectum, and those with squamous cell carcinoma of the cervix stage 3B-4A (International Federation of Gynecology and Obstetrics) should be studied. The initial studies should omit patients with squamous cell carcinoma of the esophagus (25), all lung cancers, adenocarcinoma of the pancreas, and astrocytoma (grade III-IV) (26). The known high metastatic rate, the short natural history, and/or the severe late tissue effects preclude their choice as early study sites.

The extent of the disease, tumor response to treatment, and reactions of normal tissues are reliably assessed in the recommended sites. All particle facilities (neutron, proton, and heavy ion) should participate. This would allow for testing on a parallel basis beams whose relative advantages range from presumed enhanced biologic effect to those with enhanced dose distribution capability or a combination of these.

A dose-searching study of normal tissue tolerance in the head and neck region utilizing standardized treatment delivery and standardized time-dose fractionation (RTOG Protocol 82-02) is presently planned for all US neutron users. The gross tumor and first-echelon lymph nodes will be treated by particle beam. Prophylactic neck irradiation may be delivered by x-rays or electrons. The Hammersmith dose schedule will be tested. However, in the first group of patients the total dose will be reduced by 10% as a safety measure. A total of 25-30 patients will be assessed from the participating institutions. Dose escalation will follow joint review supervised by the RTOG and the Radiation Development Branch of the NCI.

Major and minor salivary gland neoplasms, although a less significant public health issue, also deserve early testing by particle beams, given the encouraging results reported by several neutron users. The present intergroup phase III study comparing neutron-only treatment with x-rays in patients with advanced salivary gland neoplasms (RTOG 80-01, European Organization for Research on Treatment of Cancer) should be delayed until the tolerance dose schedules for head and neck squamous cell carcinoma are established.

A dose-searching study of normal tissue tolerance of pelvic organs should precede phase III testing of advanced pelvic neoplasms as well. Treating limited pelvic volumes (15 × 12 × 12 cm) by multiportal therapy now can be assessed with new equipment. It is recommended that patients be drawn from those with advanced transitional cell carcinoma of the bladder, advanced adenocarcinoma of the prostate, and recurrent adenocarcinoma of the rectum and rectosigmoid. Again, as a safety measure, the phase I-II study should begin with 90% of the Hammersmith dose.

2. Test the efficacy of pure particle beams before studying combinations of beams. Protons are an exception because of the high skin dose delivered. Their major role should be assessed as boost therapy.

3. Limit the introduction of treatment variables. The effectiveness of particles should be investigated initially independent of new developments in (*a*) adjuvant systemic chemotherapy and hormonal therapy, (*b*) radiation sensitizers, (*c*) radiation protectors, or (*d*) hyperthermia. Each particle user, however, should be permitted to use 15% of beam-time for independent investigation dependent on the special interests and resources of the facility.

4. Facilitate case accession. All patients treated under the auspices of NCI grants and contracts should be entered on RTOG protocols which are approved by the NCI. Responsibility must be borne by the institutions funded for high-LET research to make all eligible patients available for study. Each institution should operate two 8-hour shifts.

The particle effort is a program in the national interest and to ensure early completion, funds should be provided to underwrite the transportation and housing of appropriate patients at the closest particle facility.

5. Facilitate central review of protocol compliance: rapid critical review of all patients entered in the study must be conducted. Target volumes, treatment plans, and portal films should be rapidly reviewed so that necessary modifications could be reported back to the principal investigator at the start of a treatment program. Telephone transmission equipment should be available to all particle facilities and the central quality control office at the RTOG to expedite this.

6. A 1½-fold improvement in local-regional control at each test site should be looked for as the minimum criterion of effectiveness to justify this effort. Unless such an improvement in outcome is sought, prohibitively large numbers of patients would be required for phase III testing. In a two-arm study to demonstrate a 1½-fold increase in local control of tumor with a power of 0.9 and significance of $P = 0.05$, a total of 260 patients would be required (27).

7. Establish an independent high-level scientific review board composed of members not directly involved in the current clinical particle program. This board would exercise control over current operation, future planning of the program, and make final decisions on competing proposals by the institutions. It would work in conjunction with the Radiation Development Branch, NCI, which is presently empowered to review all protocols and to set priorities.

REFERENCES

1. HALL EJ, ET AL. Particle accelerators in radiation therapy. *In* Proceedings of the CROS/RTOG Part III International Workshop, Houston, Feb 1982. Int J Radiat Oncol Biol Phys 8 (12):1982.
2. SKARSGARD LD. Pion and Heavy Ion Radiotherapy, Pre-clinical and Clinical Studies. *In* Proceedings of the International Workshop, Vancouver, July 1981. New York, Elsevier Biomedical, 1983.
3. KÄRCHER KH, ROGELNICK HD, and REINARTZ G. Progress in Radio-Oncology II. New York, Raven Press, 1982.
4. KLIGERMAN MM. High-LET radiation therapy, past and future. Am J Clin Oncol 6:613-618, 1983.
5. HALL EJ. Radiobiology for the Radiologist, 2nd ed. New York, Harper and Row, 1978, pp 104-106.
6. GRAY LH, CONGER AH, EBERT M, ET AL. The concentrations of oxygen in dissolved tissue at the time of irradiation as a factor in radiotherapy. Br J Radiol 26:638-648, 1953.
7. FIELD SB. An Historical Survey of Radiobiology and Radiotherapy with Fast Neutrons, Current Topics in Radiation Research Quarterly. Amsterdam, North Holland Press, 1976, vol 11, pp 14-28.
8. HALL EJ. Radiobiology for the Radiologist, 2nd ed. New York, Harper and Row, 1978, pp 139-141.
9. FOWLER JF. Workshop summary. *In* Proceedings of the CROS/RTOG Part III International Workshop, Houston, Feb 1982. Int J Radiat Oncol Biol Phys 8:2207-2210, 1982.
10. STONE RS. Neutron therapy and specific ionization. Janeway Memorial Lecture. AJR 59:771-785, 1948.
11. BEWLEY DK, FOWLER JF, MORGAN RL, ET AL. Experiments on the skin of pigs with fast neutrons and 8 MV X-rays, including some effects of dose fractionation. Br J Radiol 36:107-115, 1963.
12. SHELINE GE, PHILLIPS TL, FIELD SB, ET AL. Effects of fast neutrons on human skin. AJR 3:31-41, 1971.
13. FLETCHER GH. Textbook of Radiotherapy, 3rd ed. Philadelphia, Lea and Febiger, 1980.
14. PETERS LJ, HUSSEY DH, FLETCHER GH, ET AL. Second Preliminary Report of the MDAH Study of Neutron Therapy for Locally Advanced Gynecologic Tumors. London, Pergamon Press, 1979. Supplement to the European Journal of Cancer, pp 3-10.
15. HUSSEY DH, MAOR MH, SAXTON JP, ET AL. Report of the M. D. Anderson Clinical Trial of Fast Neutron Therapy for Head and Neck

Cancer. Progress in Radio-Oncology II (Kärcher KH, et al, eds). New York, Raven Press, 1982, pp 111-121.

16. CATTERALL M, BEWLEY DK, and SUTHERLAND I. Second report of results of a randomized clinical trial of fast neutrons compared with X or gamma rays in treatment of advanced tumors of head and neck. Br Med J 1:1942, 1977.
17. BATTERMAN JJ. Results with a d-T Fast Neutron Machine in Amsterdam. Progress in Radio-Oncology II (Kärcher KH, et al, eds). New York, Raven Press, 1982, pp 47-56.
18. GRIFFIN T, DAVIS R, LARAMORE G, ET AL. Fast neutron irradiation of metastatic cervical adenopathy: the results of a randomized RTOG study. Int J Radiat Oncol Biol Phys 9:1267-1270, 1983.
19. DUNCAN W. Neutron beam experience in Edinburgh and other centers in Europe. *In* Pion and Heavy Ion Radiotherapy, Pre-Clinical and Clinical Studies (Skarsgard LD, ed). New York, Elsevier Biomedical, 1983, pp 345-352.
20. RAFLA-DEMETRIOUS S. Mucous and Salivary Gland Tumors. Springfield, MA, Charles C. Thomas Co, 1970.
21. SALINAS R, HUSSEY DH, FLETCHER GH, ET AL. Experience with fast neutron therapy for locally advanced sarcomas. Int J Radiat Oncol Biol Phys 6:267-272, 1980.
22. LARAMORE GE, SCHOENFELD D, HUSSEY DH, ET AL. RTOG phase I study on fast neutron teletherapy for transitional cell carcinoma of the bladder. Int J Radiat Oncol Biol Phys 8:125, 1982.
23. WHITMORE WF, ET AL. Radical cystectomy with or without prior radiation in the treatment of bladder cancer. J Urol 118:184-187, 1977.
24. VAN DER WERF-MESSING BHP. Carcinoma of the bladder $T_3N_xM_0$ treated by preoperative irradiation followed by cystectomy. Cancer 36:718-722, 1975.
25. LARAMORE G, DAVIS R, OLSON M, ET AL. RTOG phase I study on fast neutron teletherapy for squamous cell carcinoma of the esophagus. Cancer Clin Trials 5:127, 1982.
26. GRIFFIN T, DAVIS R, LARAMORE G, ET AL. Fast neutron irradiation for glioblastoma multiforme: results of an RTOG phase III randomized study. Cancer Clin Trials 5:142, 1982.
27. FOWLER JF. Doses and fractionation schemes to be employed in clinical trials of high-LET radiations. *In* High-LET Radiations in Clinical Radiotherapy, Proceedings of the 3rd Meeting on Fundamental and Practical Aspects of the Application of Fast Neutrons and Other High-LET Particles in Clinical Radiotherapy, The Hague, Netherlands, Sept 1978. Supplement to the European Journal of Cancer, 1979, pp 263-266.

Hyperthermia as a Clinical Treatment Modality

T. V. Samulski,* E. R. Lee, and G. M. Hahn[1]

The biologic rationale for using heat in the treatment of cancer is well-established. However, technical problems associated with heating human tumors have limited the clinical implementation and therapeutic evaluation of this modality. Nevertheless, the progress of hyperthermia treatment in clinical trials, although slow, has been encouraging. Two important noninvasive heating methods that are currently being used include microwave and ultrasound heating. The present limitations of these, as well as other heating methods, indicate a need for improvements in heating equipment in order to meet the challenges presented in the clinic. Fundamental aspects of heating techniques, as well as problems associated with clinical application and quantification through temperature measurements, are considered in detail in this paper.

Biologic studies carried out over the last decade have demonstrated that the use of mildly elevated temperatures has cytotoxic properties which are useful in the treatment of cancer, particularly in the treatment of solid tumors (1). Such studies have shown that low pH, nutrient depletion, and chronic hypoxia all tend to sensitize cells to the action of heat. These features characterize the extracellular milieu in parts of most solid tumors. Additionally, studies have indicated that at least some malignant cells inherently tend to be more heat-sensitive than their normal counterparts. It is not known if this is a general proposition; however, if only a reasonable proportion of malignant cells are heat-sensitive, this would be sufficient to warrant the introduction of hyperthermia into the clinic.

Heat has other properties that make it attractive as a treatment modality. During x-irradiation, heat increases the effectiveness of the ionizing radiations (2). Heat does not have to be given simultaneously with radiation to show synergistic effects; it is sufficient that the two treatments follow each other closely in time, with no more than a few hours intervening. In fact, heat may be the best sensitizer of both oxygenated and hypoxic radioresistant cells that has been discovered to date.

Heat also increases the effectiveness of many chemotherapeutic agents (3,4). The cytotoxic effects of the nitrosoureas, cisplatin, bleomycin, etc, are all vastly enhanced at temperatures of $\geqslant$ 43°C. The combination of low pH and elevated temperatures can even further increase the cytotoxicity of some drugs. Thus, hyperthermia appears to be a "triple threat."

Despite these benefits, hyperthermia is only slowly finding its way into the clinic. It can be said with some assurance that radiotherapists and surgeons have almost exclusively become its advocates. The most optimistic proponents of hyperthermia admit that clinical results to date, while certainly encouraging, have not fulfilled initial expectations. Why should this be? As might be expected, this is a complex question and there is no simple answer. Some of the contributing reasons are that the optimum fractionation protocols, either with hyperthermia alone or in conjunction with radiation therapy or chemotherapy, have not yet been worked out. Most therapists tend to be cautious with this new technique, and treatment to maximum tolerance is rarely, if ever, used. The maximum tolerance to heat of most normal tissues has not yet been measured in animal systems. In most clinical studies, the patients treated had far-advanced cancers, and phase I studies of this type do not yield data on clinical efficacy. Despite that, it must be pointed out that the results obtained to date are not that discouraging. From table 1, it can be seen that the combination of heat and radiation therapy is appreciably superior to the use of radiation therapy alone. It is doubtful that there are any chemotherapeutic agents claiming equivalent successes at this early stage.

The dominant reason why hyperthermia has not lived up to the promises raised by the biologic experiments is the technical difficulty of elevating tumors to appropriate temperatures and determining the temperature distributions obtained. Yet, heating arbitrary volumes is theoretically feasible. To a large extent, the problems persist because individuals working in this area do not appreciate the difficulties associated with adequately heating tumors. Hence, there is an insufficient effort in developing appropriate equipment. Historically, this situation has come about because experiments on mice do not require sophisticated heating equipment. In such ex-

---

[1] Department of Radiology, Stanford University, CA.

**Reprint requests to:* Thaddeus V. Samulski, PhD, Stanford University Medical Center, Rm S-044, Stanford, CA 94305.

TABLE 1.—Responses of patients with matched superficial lesions to radiation and radiation + heat[*]

Investigator (yr)	Tumor histology	No. of patients	% of patients with radiation alone/response[†]	% of patients with hyperthermia + radiation/ response[†]
Kim et al (1979)	Various	54	26/CR	78/CR
Kim et al (1982)	Malignant melanoma	38	46/CR	70/CR
Marmor et al (1980)	Various	15	7/Superior	47/Superior
U et al (1980)	Various	7	14/CR	86/CR
Archangeli (1980)	Neck node metastases from head and neck primary sites	15	46/CR	85/CR

[*] Patients with multiple metastatic superficial tumors had 1 lesion treated with radiation alone and 1 lesion treated with hyperthermia + radiation.

[†] CR = complete response; superior = CR vs partial response, or > 6 wks difference in time to regrowth.

periments, the investigator is able to place the tumors in locations that are easily accessible, treat the tumors when they are of a size that makes uniform heating possible, and make tumor temperature measurements without great difficulty. Therefore, complex heating equipment is not required. However, the situation for humans is very different. Most tumors are of irregular shape, they are removed from the surface of the body, and their dimensions vary from a few cubic centimeters to large regions occupying diverse regions of the anatomy. Sophisticated equipment is necessary to heat such lesions uniformly. In the absence of noninvasive methods of measuring temperature, human tumor temperatures can, at best, be sampled at only a few locations and, in most situations, adequate mapping is impossible. Treatment failures in the clinic may be associated with the existence of one or two small cold spots within the tumor volume. Therefore, the difficulties resulting from lack of adequate knowledge of tumor temperatures can readily be appreciated.

In this paper, we will discuss some of the techniques used to heat tumors and measure temperature and will concentrate on clinical problems involving their usage.

Methods of Inducing Hyperthermia

Most of the methods that depend on conduction of heat through tissue are useful only for heating very superficial lesions. Such techniques include hot water baths, local application of heated elements, and infrared radiation.

For heating more deep-seated lesions, two approaches are feasible. The first approach is to place energy sources in the immediate vicinity of the tumor, which limits heating to the tumor volume. Such invasive techniques are particularly useful in conjunction with brachytherapy, where much of the same equipment used for implanting radioactive material can also be used for heating (5). The other approach is to attempt to heat noninvasively, usually by passing either an ultrasound or an electromagnetic wave through the tumor or by attempting to regulate electric current flow so that it will heat the tumor. With all of the noninvasive approaches, excessive heating of normal tissue is the most serious problem to be addressed. The invasive approaches are limited in a different way; we will not discuss them in detail here, but will concentrate on methods employing noninvasive microwaves and ultrasound.

Microwave Heating

The techniques for inducing hyperthermia via electromagnetic (EM) fields are quite diverse and it is important to distinguish fundamental differences. There is a traditional nomenclature (radiofrequency [RF], microwave, etc) assigned to the EM frequency spectrum. However, in clinical hyperthermia, the distinction between RF or shortwave and microwave heating is not of EM frequency as much as it is of whether the EM energy deposition in tissue is predominantly from nonradiating or radiating sources. In all cases, the absorbed energy density at a point in exposed tissue is proportional σE^2, where σ is the tissue conductivity at the EM field frequency and E is the magnitude of the electric field at the point of interest. Both RF (ie, capacitive and inductive heating) and microwave techniques can be invasive and noninvasive and can be used in clinical hyperthermia treatments. However, our main concern here will be with microwave heating, which is one of the more commonly used methods.

There are two potential advantages in using microwave radiation fields for hyperthermia. First, it is a noninvasive technique, and this aspect is generally desired in clinical practice. Second, because of the continuity conditions of the EM field at boundary interfaces, a propagating transverse EM field, with its electric and magnetic field components parallel to a tissue interface (ie, fat/muscle), can deposit energy in the deeper tissue (muscle) without selectively heating the more superficial tissue (fat). The problems of selective superficial tissue heating are clinically significant but not completely solvable by using a transverse propagating EM field, since the incident wave is not always normal to the boundary interface. Nevertheless, the propagating EM wave is generally an improvement over capacitive RF heating with regard to sparing superficial fat tissue.

The interaction of the microwave field with tissue is governed by the size, shape, and electric properties of the tissue (the relative dielectric constant, ε, and the conductivity, σ). With regard to the electric properties, tissues can generally be classified into two types: (*a*) tissues with high water content (eg, skin, muscle) and (*b*) tissues with low water content (eg, fat, bone) (6,7). The values of the relative dielectric constant and conductivity depend on EM frequency and differ by about an order of magnitude between the two tissue types. The properties of specific tissues have been measured both in vitro and in vivo over a wide frequency range and have been reported in the literature (6-9). Such detailed data are required to accurately determine or predict the power absorbed or specific absorption rate in body regions when exposed to microwave radiation. At present, there is not sufficient data on human neoplasms to indicate a preferred frequency which would allow any degree of selective heating in tumor, rather than normal, tissue. It is not expected that such a differential, if it exists, would be more important than other effects, such as local perfusion, in determining how successfully a target volume can be heated.

The heterogeneous distribution of tissue types through the anatomy and corresponding irregular geometries present formidable difficulties in predicting the exact microwave interaction and consequent energy deposition. This task of predicting absorbed power distributions will require high-level numeric techniques, a level of sophistication not yet available clinically. The sensitivity of the absorbed energy distribution to tissue types and geometry can be appreciated by examining the theoretic solutions for some simple models. Figure 1 shows the results of analysis of an EM plane wave source at normal incidence on a layered fat/muscle model (10). The penetration profile for absorbed energy is frequency-dependent, with lower frequencies having greater penetration depth. The relative amounts of energy deposited in the fat and muscle tissues are also frequency-dependent and depend on the thickness of the fat layer as well. If the fat layer is thicker than one-quarter wavelength, it is possible to have a standing wave hot spot. This standing wave condition is the result of an interference pattern established between the incident EM wave and the reflected EM wave, which is shifted 180° in phase at the boundary interface. For the case of 2450 MHz, this hot spot can occur in a fat thickness of < 2 cm, which is a condition easily met in patients having clinical hyperthermia treatments. Other models which have been theoretically approached include heating spheric and cylindric tissue models with plane wave sources (10,11). The results show that because of the dielectric discontinuity and geometric curvature, irradiation with wavelengths larger than the radius of curvature has a focusing effect which can increase the central energy absorption by several orders of magnitude over that expected from simple plane wave attenuation. Since body contours or protruding lesions often fall within this curvature wave length criterion, field focusing is important in determining EM-absorbed power distributions.

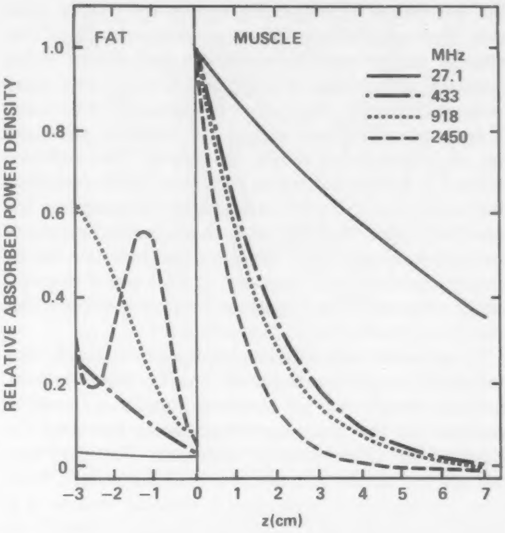


FIGURE 1.—Calculated relative power density in fat and muscle layers exposed to a plane wave source at normal incidence on the fat layer (ref 10).

There are two advantages in using lower frequency microwaves: (*a*) greater penetration and (*b*) better muscle-to-fat heating profiles. However, in practice, there are trade-offs for lowering frequency, which are a loss in local power deposition and an increase in physical dimensions of the EM source applicator. These limitations are so severe that "local" microwave heating is only practical in the frequency range of 300-1000 MHz and is restricted in depth to about 2 or 3 cm.

Except for the radiating applicator or antenna and special thermometry system, the components of the superficial microwave hyperthermia system are readily available. This equipment includes a generator capable of continuous power output, which is controllable either manually or remotely in steps of 1-2 watts to a maximum of 200 watts. This power source needs to be stable in frequency and power output for periods of prolonged use (4-5 hours) and should be isolated from reflected power. A power-monitoring capability with resolution and accuracy of 1-2 watts is necessary for measuring forward and reflected power between the isolated generator and radiating applicator. Since temperature data during treatments are usually minimal, the net power (forward minus reflected) is an important parameter in governing the efficacy and safety of treatments.

The generator and radiating applicator are usually connected via a coaxial transmission line. For power to be efficiently transferred, the electrical impedance should be matched at the generator/transmission line and the transmission line/applicator interfaces. The generator and line are usually matched to a 50 ohm standard. However, the applicator impedance is variable, because it is highly dependent on the detailed coupling between the applicator and tissue. This necessitates an impedance matching or tuning network in the system. The tuning can be accomplished in a variety of ways, but usually consists of a combination of adjustable tuning stubs.

By far, the heating applicators are the weak length in current superficial microwave systems. The most effective applicators currently available are the waveguide and resonant cavity types. These devices are designed to operate with a small intervening air gap or in direct contact through a coupling medium such as deionized water. In general, the direct contact applicators are more efficient in transferring EM energy into the tissue, with minimum stray leakage. Also, circulating a thermostatically controlled coupling fluid is very useful for regulating skin temperature, which is an important treatment consideration.

For an applicator to radiate effectively, its physical dimensions must be related to the radiation wavelength (ie, on the order of one-half wavelength). This constraint can be circumvented to some extent through design (eg, ridged waveguides) or by reducing the effective wavelength by loading the waveguide or cavity with a low-loss dielectric material. (The dimensions are reduced by

$$\frac{1}{\sqrt{\varepsilon}}$$

where ε is the dielectric constant of the loading material.) An extreme example of this type of loaded applicator is a ridge waveguide which operates at 27 MHz (12). The free-space wavelength at 27 MHz is 11 meters, whereas the dimensions of the reported waveguide are approximately 1 meter. Other examples of unique hyperthermia microwave applications have also been reported (13). It is worth noting that forcing longer wavelength EM fields into smaller dimension waveguide-type applicators does not buy the gain in penetration depth that one would expect from a plane wave source. One reason is the present additional EM field modes, other than the desired fundamental transverse electric field, which are damped out in the course of propagation through the guide. If the guide is not sufficiently long, these "near field" components can extend into the spatial regions near the applicator aperture. The rapid attenuation of these components contributes to the diminished penetration depth. "Near field" patterns are difficult to calculate, particularly in the presence of a lossy dielectric such as tissue. They can have significant longitudinal components and again allow the possibility of selective heating of superficial fatty tissue.

A drawback associated with currently available microwave heating applicators is the fixed EM field distribution across the radiating aperture, which has a maximum field in the center falling off to essentially zero at the edges. In the simplest rectangular waveguide operating in the dominant mode (T_{10}), the power distribution falls off like a

$$\cos^2(\frac{x\pi}{d})$$

function from center ($x = 0$) to edge ($x = d/2$). The consequence of this energy profile is that the effective heating area is centrally located and much smaller than the physical aperture. The distribution is even more sharply peaked when ridged designs are used (fig 2); thus, a trade-off in applicator size and effective heating field is evident. The centrally concentrated field distribution is a disadvantage that is often exacerbated when clinically heating nodules with poorly perfused central cores. The tumor center is often heated to very high temperatures (> 45 °C), while the peripheral interface between tumor and normal tissue is left at less than therapeutic temperatures (fig 3).

Some efforts are being made to design microwave heating applicators which are more capable of handling the problems of body contour, tissue, and blood flow inhomogeneities. These efforts include the development of microstrip radiators, which have some degree of contour conformation (14-16). These radiators can be small enough to arrange in a multiple antenna array, which will allow manipulation of absorbed energy profiles to compensate for local perfusion (17).

As previously mentioned, except for very low frequencies, microwave penetration into tissue is limited to superficial heating. There are possibilities for effective heating at greater depths by using fixed arrays of radiators, so that the EM fields from individual radiators add coherently at depth. Since the power absorbed is proportional to the square of the electric field, a phase array can, in principle, enhance the energy deposition of a single radiating source by the square of the number of

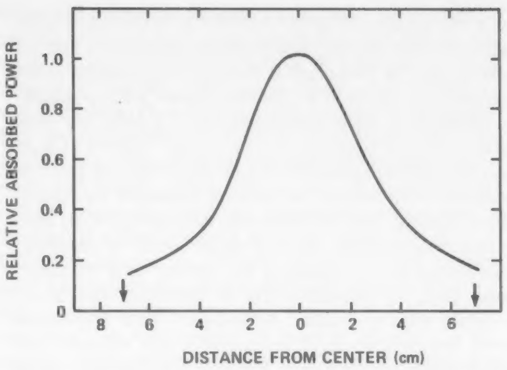


FIGURE 2.—Absorbed energy profile for a ridged waveguide applicator operated at 390 MHz. Profile is measured in a tissue equivalent phantom at 4-cm depth (2-cm fat, 2-cm muscle). Arrows indicate the physical dimensions of the applicator aperture.

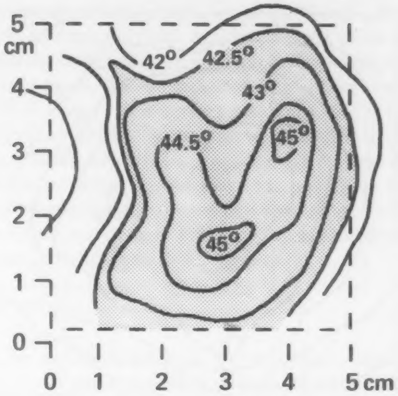


FIGURE 3.—Temperature profile measured at 1-cm depth in a tissue plane parallel to the skin surface during a superficial microwave treatment. Dashed boundary indicates the prescribed treatment area.

sources used in the array. This principle has been employed in one device, the annular phased array system, which is designed for deep regional heating (18). This system is configured so that a fixed hexagonal annulus containing 16 microwave radiators on the periphery is operated in a phase-coherent manner to create a concentrated energy distribution in the central region of the annulus. With the system, enhanced central energy distribution exists when the body trunk is within the annular aperture and coupled to the radiating antennae through a deionized water bolus. At the present time, this system is still experimental and is being evaluated clinically for efficacy in deep regional heating.

A necessary element in clinical microwave hyperthermia is a temperature monitoring system. Artifactual heating and field perturbation problems associated with metallic thermocouple and thermistor temperature probes placed in EM fields can be avoided, since there are several clinically usable nonperturbing thermometry systems now available. These include the high-resistance lead thermistor probes and other systems which use optical techniques with fiber optic probes (19–21). The resolution and accuracy obtainable with these systems are approximately 0.1 °C. The physical diameters are approximately 1 mm, making them suitable for tissue implants.

The outstanding problem with thermometry in hyperthermia stems from its invasive nature, and there is no readily foreseeable solution to this constraint. Only a limited amount of temperature data can be obtained clinically with invasive probes. However, there are improvements possible with the development of sensor arrays, which will have several appropriately spaced temperature sensors on a single probe (21). These will allow maximum temperature monitoring with minimum tissue invasion.

Using the thermometry system to provide feedback control for the power generator is a straightforward step in an integrated heating system (22). The requirements of a fail-safe control system can be demanding for the dynamic situation present in hyperthermia, and more clinical experience is needed before flexible control algorithms can be developed which allow optimized heat treatments.

Ultrasound Heating

There is a fundamental difference between the ways the EM and ultrasound waves transfer energies to tissue. The former consists of a field propagating either through a vacuum or a medium and existing, in a sense, independently of the environment. Energy transfer involves interaction between charged elements of atoms (primarily electrons) and the EM field. However, sound is a compression wave and, as such, requires the presence of matter. Sound does not propagate through a vacuum. The transfer of energy is to mechanical systems. Differences in the mechanical properties of the medium become very important. For example, absorption of energy by bone is very different from that by soft tissue. The velocity of propagation of sound in any biologic tissue is appreciably slower than that of EM frequencies, and the consequent wavelength is much smaller. The big advantage of sound that results from short wavelengths is that beam shaping is quite feasible and achievable at the frequencies of interest. Although so-called "near-field" effects (the energy distribution occurring in the neighborhood of the transducer) are present, regions of high- and low-energy deposition are closely spaced, and hot and cold spots are generally "smeared out" by conduction. Thus, relatively uniform treatment fields can be obtained with ultrasound in tissue (fig 4).

The useful ultrasound frequency range for clinical

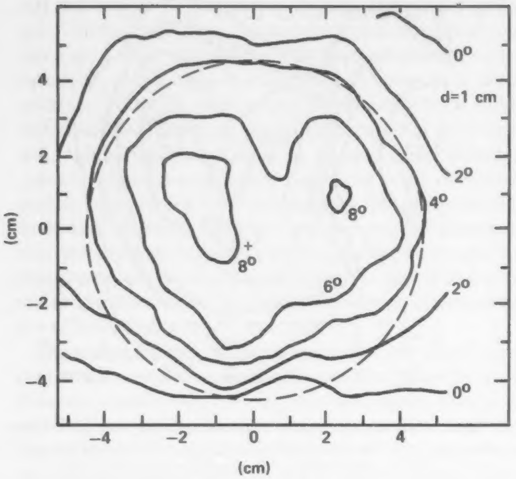


FIGURE 4.—Temperature elevation isotherms measured in vivo (pig thigh). Transducer is operated at 1.066 MHz with +35 kHz frequency modulation. PZT-5 ceramic crystal applicator position and size are indicated by the dashed line. Depth in tissue is 1 cm.

hyperthermia, as dictated by absorption considerations, is from about 300 kHz to 3 MHz. At the upper end of the frequency scale, attenuation limits penetration to about 1 cm of tissue. In most soft tissue, attenuation is approximately 1 db/MHz cm; in bone, it can be an order of magnitude higher (23). Thus, treating tissue overlying bone frequently causes pain, and the large reflection coefficient at bone-tissue interfaces may produce standing wave patterns that result in very high localized heating. Again, the high attenuation rate and precise energy deposition associated with the high-frequency sound can minimize this problem.

Using ultrasound for the treatment of superficial lesions at high powers and frequencies presents several technical difficulties. The normal operation of a piezoelectric ceramic crystal for ultrasound power generation is at the first harmonic resonance, which requires the thickness of the disk to be equal to one-half of the ultrasonic wavelength. At higher frequencies, the thickness of the crystal may well be < 1 mm. (For PZT-5, a piezoelectric material frequently used, the thickness is 0.6 mm at 3 MHz.) Since the crystal's diameter may have to be large in order to heat a sufficient area (10 cm), this small thickness-to-diameter ratio causes the material to be very brittle and malfunctions of the crystal are difficult to avoid. Another problem is that the electric coupling becomes increasingly difficult at high frequencies. This is because the dielectric constant of the materials used is in the range of 1000-2000, so that the capacitive reactance at these frequencies becomes appreciable.

When going down in frequency, ultrasonic absorption is less and the penetration depth increases. For most tissues, the amount of power required to achieve and maintain sufficient heating is somewhere between 10 and 100 mW/cc. As the frequency is reduced, the incident power required to achieve this absorbed power goes up rapidly. In addition, at lower frequencies, ie, < 1 MHz, the near-field structure becomes coarser, so that regions of high- and low-power deposition become separated and their importance with respect to causing excessive (or insufficient) heating must be taken into consideration. To some extent, this problem can be reduced either by frequency modulation or by operating the crystal at a frequency away from its resonance (fig 5). However, this is not achieved without penalty. A crystal driven at frequencies away from its resonant frequency is less efficient, and drive power requirements increase even further. Additionally, characteristics of the "near-field" pattern become more difficult to predict.

Ultrasound beam shaping can be achieved in a variety of ways. The focusing of a single transducer can be accomplished by shaping the crystal or adding an acoustic lens. Multiple transducer units using either focused or unfocused individual transducers have been designed to produce preferential energy deposition at depth (23,24). Dynamic systems can also accomplish this by mechanically moving one or more transducers or by varying electronically the phasing between transducers in a predetermined fashion (25,26). The potential for use of such techniques at the lower end of the cited frequency range gives

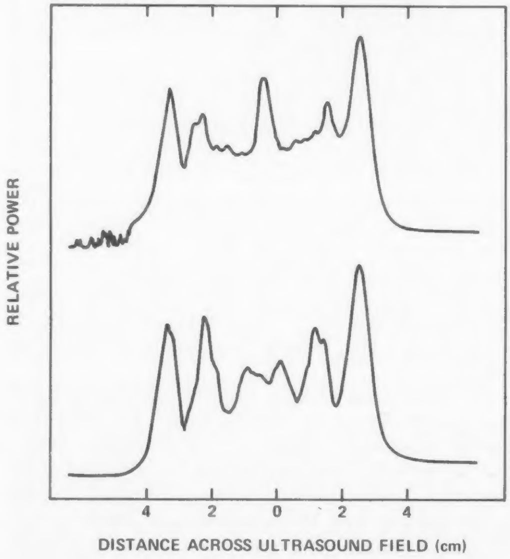


FIGURE 5.—Ultrasound near-field power profiles taken in a water tank with an 8.5-cm clear aperture transducer. In the lower scan, the operation is without frequency modulation. In the upper scan, the operation is at 1.066 MHz with +35 kHz frequency modulation.

ultrasound unique advantages: maximum energy deposition can be determined quite precisely, absorption is not excessive, and preferential heating of deep-seated tissue volumes is feasible. This can be done more efficiently with ultrasound than with any other available technique. Why, then, is ultrasound not used more frequently? The answers are: first, lack of available commercial equipment; and second, limitations arising from anatomical considerations. In a multiple transducer system that is arranged in a fixed manner, there are relatively few anatomical regions with a surface area large enough for coupling the transducers without an obstruction of their energy flow by intervening bone structures or tissue-air interfaces. To some extent, at least in principle, a dynamic system can bypass these problems. If scanning is under microprocessor control, then, by appropriate motion of the transducer, bone surfaces can be avoided and energy passage oriented so that preferential heating of tumors is possible. First-generation equipment based on fixed arrays and scanning techniques has been developed and is currently being clinically evaluated (1,25,26). It is in these areas that a large effort in equipment design could pay off handsomely in terms of clinically useful equipment.

Thermometry in an Ultrasound Field

The problems associated with temperature measurements in ultrasound fields are almost the inverse of those in EM fields. Small metal inserts are not preferentially heated by ultrasound, nor does ultrasound induce noise in a well-constructed thermocouple or thermistor. In general, thin metal objects cause few or no problems. On the other hand, rubber or plastic-like materials may absorb ultrasound very differently from adjacent tissue and heat up preferentially. This is a problem that must always be considered. Materials bonding a thermistor to a hypodermic needle may cause preferential absorption and result in erroneous temperature readings. In clinical treatments, we frequently attempt to obtain temperature data over one-linear dimension by inserting a catheter into the tumor and measuring temperature along the catheter by physically moving the sensor. In the case of an ultrasound field, this becomes very difficult to do. Catheters and insulating sheaths made of polyvinyl chloride, nylon, teflon, silicone rubber, neophrene rubber, latex, and parylene have been tested both in vivo and in vitro. In all cases, we have observed preferential absorption resulting in localized heating. Plastic materials used in optical temperature probes have also shown artificial heating during ultrasound irradiation. Even very thin layers of absorbing materials can cause appreciable temperature measurement errors. In one of our tests, a multijunction flexible thermocouple probe array consisting of copper-constant wires insulated with vapor-deposited parylene, with thickness between 30 and 70 μm, showed temperature artifacts in vivo varying between 0.4 °C and 1.5 °C in a 360-kHz ultrasound field. An uninsulated probe that was similarly tested showed no temperature artifact at equivalent powers. These errors are in addition to those that are inherent in any system involving heat conduction along the wires of the measuring system. It is possible to bypass many of these problems by making thin-walled metallic catheters. An alternate method is to insert uninsulated thermocouples or thermistors into tissue. The latter approach is limited by mechanic durability, biocompatibility, and electric safety.

CONCLUSION

We have discussed some of the problems associated with the use of microwave and ultrasound equipment in the clinic. Although we have focused on the difficulties with these methods of treatment, the use of inductive or capacitive so-called radiofrequency heating has problems that may be somewhat different, but equally serious. We have also shown that, in addition to heating problems, thermometry considerations must always be kept in mind and these may take on appreciable dimensions.

At this point, the reader may think that technical problems associated with heating are so overpowering as to make hyperthermia hopeless. We do not want to give such an impression. If, at the time of Roentgen, anyone had considered the technical problems associated with deep-seated radiation therapy, the problems would have appeared far more formidable than those associated with heating today. In fact, it may be useful to consider the comparison between radiation therapy and hyperthermia. One might begin with dosimetry. In the case of radiation therapy, there are no attempts of actual measurements of energy deposited in human tissue. Calibrations of the output and shape of the radiation beam are done routinely, but energy deposition is calculated and measurements are routinely deemed unnecessary. The reason why such calculations are acceptable for radiation therapy is that careful measurements of tissue absorption have been made. The interaction of tissue with radiation has been carefully established and beam shape characteristics have been carefully measured. Furthermore, treatment planning is possible because the limits of tolerance of various tissues to radiation have been established empirically. As yet, none of this applies to hyperthermia. Although many investigators are developing techniques for predicting temperature distributions based on the bioheat equation, such techniques are not yet used in the clinic. The complexities associated with the predicted techniques are more difficult with heat than with radiation, largely because of blood flow. Blood flow patterns are difficult to incorporate in mathematic models; furthermore, blood flow rates are dynamic and may change during hyperthermia treatment. Perhaps if the nuclear magnetic resonance imaging is added to information obtained from computerized tomographic scans, it may be possible to model treatment planning in hyperthermia. When confidence in the modeling system has been gained (eg, in animal tests), invasive thermometry may no longer be necessary.

Much of the same discussion can be made about heat delivery. When x-rays were first developed, absorption characteristics of the low-energy x-rays available prevented penetration to deep-seated sites. It was not until the development of megavoltage equipment (cobalt and linear accelerators) that adequate irradiation of deep-seated tumors became feasible. However, even with the best of external equipment, many tumors respond better to implants and, therefore, invasive techniques are still being used. Tolerance to x-ray of normal tissue was totally unknown. With respect to heating equipment and its use, we are in a very similar situation today. Penetration is limited by absorption characteristics. This is particularly true for the use of EM energy. Well-controlled beam focusing is only possible for ultrasound, and the normal tissue damage (in this case, mainly bone) is limiting.

Within the next few years, advances in hyperthermia research should indicate that physics and biology will be in balance to the point where adequate heating of tumors is possible, and sufficient knowledge regarding temperature distributions can be obtained. Only when this happens can the full clinical potential of hyperthermia be evaluated.

REFERENCES

1. HAHN GM. Hyperthermia and Cancer. New York and London, Plenum Press, 1982.
2. WESTRA A, and DEWEY WC. Heat shock during the cell cycle of Chinese hamster cells in vitro. Int J Radiat Oncol Biol Phys 19:467-477, 1971.
3. HAHN GM. Potential for therapy of drugs and hyperthermia. Cancer Res 39:2264-2268, 1979.
4. MARMOR JB. Interactions of hyperthermia and chemotherapy in animals. Cancer Res 39:2269-2276, 1979.
5. MANNING MR, and GERNER EW. Interstitial thermoradiotherapy. *In* Hyperthermia in Cancer Therapy (Storm FK, ed). Boston, GK Hall and Co, 1983.
6. SCHWAN HP, and PIERSAL GM. The absorption of electromagnetic energy in body tissues: part I. Am J Phys Med 33:371-409, 1954.
7. ______. The absorption of electromagnetic energy in body tissues: part II. Am J Phys Med 34:425-448, 1955.
8. STUCHLY MA, KRASZEWSKI A, STUCHLY SS, ET AL. Dielectric properties of animal tissue in vivo at radio and microwave frequencies: comparison between species. Phys Med Biol 27:927-936, 1982.
9. KRASZEWSKI A, STUCHLY MA, STUCHLY SS, ET AL. In vivo and in vitro dielectric properties of animal tissue at radio frequencies. Bioelectromagnetics 3:421-432, 1982.
10. JOHNSON CC, and GUY AW. Nonionizing electromagnetic wave effects in biological materials and systems. *In* Proceedings of the Institute of Electrical and Electronic Engineers (IEEE) 60:692-718, 1972.
11. HO HS, GUY AW, SIGELMANN RA, ET AL. Microwave heating of simulated human limbs by aperture sources. IEEE Trans Microwave Theory and Tech MTT-19:224-231, Feb 1971.
12. PAGLIONE R, and STERZER F. 27 MHz ridged waveguide applicators for localized hyperthermia treatment of deep-seated malignant tumors. Microwave J 24:71-80, 1981.
13. KANTOR G. Evaluation and survey of microwave and radiofrequency applicators. J Microwave Power 16:135-150, 1981.
14. BAHL IJ, STUCHLY SS, and STUCHLY MA. A new microstrip slot radiator for medical applications. IEEE Trans Microwave Theory and Tech MTT-28:1464, 1980.
15. SANDHU TS, and KOLOZSVARY A. Effects of bolus/tissue inhomogeneities on the resonance frequency of microwave microstrip applicators. *In* Proceedings of the North American Hyperthermia Group (NAHG), San Antonio, TX, 1983.
16. STERZER F, PAGLIONE R, NOWOGRODZKI M, ET AL. Microwave apparatus for the treatment of cancer. Microwave J 23:39-44, 1980.
17. TANABE E, MCEUEN A, NORRIS C, ET AL. A multi-element microstrip antenna for local hyperthermia. IEEE MTT-5. *In* Proceedings of the International Microwave Symposium Digest, Boston, May 31-June 3, 1983.
18. TURNER PF. Deep heating of cylindrical or elliptical tissue masses. *In* Proceedings of the Third International Symposium: Cancer Therapy by Hyperthermia, Drugs and Radiation, Fort Collins, CO, June 1980. NIH Publ No. 82-2437, pp 493-495.
19. BOWMAN RA. A probe for measuring temperature in RF heated material. IEEE Trans Microwave Theory and Tech MTT-27:673, 1979.
20. WICKERSHEIM KA, and ALVES RB. Recent advances in optical temperature measurement. Ind Res Dev 21:82, 1979.
21. VAGUINE VA, CHRISTENSEN DA, WALSTON TE, ET AL. Multiple sensor optical thermometry system for application in clinical hyperthermia. *In* Proceedings of the North American Hyperthermia Group, San Antonio, TX, 1983.
22. CHUNG AY. Microwave techniques for clinical hyperthermia. *In* Physical Aspects of Hyperthermia (Nussbaum GH, ed). American Association of Physicists in Medicine (AAPM) Monogr No. 8, 1982.
23. WELLS PNT. Physical Principles of Ultrasound Diagnosis. London and New York, Academic Press, 1969, p 24.
24. HAYAKAWA Y, EGAWA J, ISHIOKA K, ET AL. Characteristics of localized ultrasonic field formed by an apparatus using five planar PZT crystals. *In* Proceedings of the Third Hyperthermia Seminar, Kyoto, Japan, Oct 1980.
25. DO-HU JP, and HARTMANN P. Deep and local heating induced by an ultrasound phased array transducer. *In* Proceedings of the 1982 Institute of Electrical and Electronic Engineers (IEEE). Ultrasonics Symposium, San Diego, vol 1, 1982.
26. LELE PP. Physical aspects and clinical studies with ultrasound hyperthermia. *In* Hyperthermia in Cancer Therapy (Storm FK, ed). Boston, GK Hall and Co, 1983, pp 333-365.

Diagnosis of and Therapy for Solid Tumors With Radiolabeled Antibodies and Immune Fragments

Jorge A. Carrasquillo,[1,2,3] Kenneth A. Krohn,[2] Paul Beaumier,[4] Robert W. McGuffin,[1] Joseph P. Brown,[4] Karl Eric Hellström,[4] Ingegerd Hellström,[4] and Steven M. Larson[1,2,3,*]

Antibodies which are directed against human tumor-associated antigens can potentially be used as carriers of radioactivity for in vivo diagnosis (radioimmunodetection) or treatment (radioimmunotherapy) of solid tumors, including colon, hepatoma, cholangiocarcinoma, and melanoma. Murine monoclonal antibodies (MOAB), produced by the hybridoma technique of Kohler and Milstein, are replacing conventional heterosera as sources of antibodies, because MOAB can be produced in large quantities as reproducible reagents with homogeneous binding properties. We have studied human melanoma using MOAB IgG and Fab fragments that recognize the human melanoma-associated antigens p97 and "high-molecular-weight antigen." Both antigens are found in the membrane of melanomas at much larger concentrations than in normal adult tissues. We have performed radioimmunodetection studies with whole immunoglobulin and have detected 88% of lesions > 1.5 cm. We have used Fab fragments for radioimmunotherapy and have found that large doses of radiolabeled antibodies (up to 342 mCi) can be repetitively given to patients without excessive end-organ toxicity. Two of three patients treated with high-dose radiolabeled antimelanoma Fab showed an effect from the treatment. Although both technical and biologic problems remain, the use of radiolabeled antibodies that are directed against tumor-associated antigens holds future promise as a new therapeutic approach to solid tumors that are resistant to conventional therapy. **[Cancer Treat Rep 68:317–328, 1984]**

Recent advances in tumor immunology have led to the availability of antibodies which are immune-specific for tumor-associated antigens on common human tumors, including solid tumors [colon (1), breast (2), hepatoma (3), melanoma (4), and germ cell tumors (5)]. We and others are exploring a diagnostic and therapeutic strategy whereby these tumor-specific antibodies are used as carriers of radiolabeled isotopes for in vivo localization of radiation to tumors. The underlying principle is that when these radiolabeled antibodies are introduced into the bloodstream of patients with certain tumors, the antibodies are passively carried to the tumor, where they specifically bind to tumor at tumor-antigen binding sites. The antibodies bypass the normal tissues, because there is no specific antigenic binding sites in these tissues. Gradually, over the course of several hours to a few days, a progressively greater and greater concentration of radiolabeled antibody accumulates in the tumor as compared to normal tissue. When tracer doses of radiation are used to label the antibodies, the location of metastases can be detected using nuclear medicine imaging techniques; this general process has been termed "radioimmunodetection" (6). When larger amounts of radiation are employed, antitumor therapy is possible; this process is termed "radioimmunotherapy."

HISTORICAL PERSPECTIVE

Pressman (7) was among the first to explore the use of radiolabeled antibodies for tumor localization. This investigator invented a simple but elegant technique to demonstrate that antigen-specific binding in tumor is responsible for the localization of radioactivity in vivo after tumor-specific radiolabeled antibodies are injected into experimental animals. Two antisera, one obtained after immunization with a rat sarcoma and one obtained simply after bleeding a nonimmune rabbit, were labeled with ^{131}I and ^{125}I, respectively. After iv injection into several rats bearing Wagner osteogenic sarcomas, a period of localization was permitted and the rats were then killed. When the tumors were removed and counted for radioactivity, it could be shown conclusively that significantly more radioactivity was borne to the tumor by the immune antibody. A specificity ratio (radiolabeled specific antisera/radiolabeled nonspecific antisera) was calculated by Pressman, and for his early studies, this ratio was about 2. Nonetheless, this degree of specificity was not sufficient for human studies, and progress toward greater specificity was retarded. Two problems which interfered with progress in this field were the lack of tumor-specific antigens and the lack of well-defined and reproducible antibodies.

---

[1] Nuclear Medicine Section, Veterans Administration Medical Center, Seattle, WA.

[2] Division of Nuclear Medicine, University of Washington, Seattle.

[3] *Present address:* Nuclear Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD.

[4] Program of Tumor Immunology, Fred Hutchinson Cancer Research Center, Seattle, WA.

[*] *Reprint requests to:* Steven M. Larson, MD, Nuclear Medicine Department, Clinical Center, National Institutes of Health, Bldg 10, Rm 1C401, Bethesda, MD 20205.

Using immunologic techniques, Gold and Freedman (1) demonstrated in 1965 that human colon tumors contained a tumor-specific antigen, the carcinoembryonic antigen (CEA). Moreover, they showed that anti-CEA antibodies (obtained from heterosera) could be used in radioimmunoassays of patient sera to detect patients with colon cancers. The presence of the human tumor antigen and the diagnostic usefulness of antibodies developed against this antigen kindled the interest of scores of tumor immunologists to search for antigens in other tumors as well. Several such antigens were found, including alpha-fetoprotein (8) and some melanoma-associated antigens. Actually, these antigens were not truly tumor-specific; they were merely inappropriate production of gene products which were not seen in most normal adult tissues. In general, the fetus produced these biologic molecules. Thus, the tumor-associated antigens were in effect "oncofetal" biomolecules. Even today, most of the tumor-associated antigens commonly recognized on solid tumors are in fact oncofetal substances and are not exclusive to tumor. In some instances, normal tissues may express small amounts of antigens.

Although isolation of exclusively tumor-specific antigens still eludes us, considerable progress in identifying and characterizing several tumor-associated antigens has been made. For the purposes of radioimmunodetection or therapy, these tumor-associated antigens must be expressed in higher concentration in tumor than in normal tissue.

Radioimmunodetection With Heterosera

Beginning in the late 1970s, several investigators began to explore the possibility of using tumor-specific heterosera for the purposes of radioimmunodetection, and initial studies performed in animals demonstrated the feasibility of using them for imaging. In humans, external scintigraphy has been used to image human cancers, with antibodies specific for CEA (6,9,10), human chorionic gonadotropin (11), alpha-fetoprotein (12,13), kidney carcinoma (14), and ferritin (15). Initial reports cited a diagnostic accuracy of about 85% (6). Image-enhancement techniques (6) and absorption of xenosera (16) improved sensitivity. Nonetheless, the amount of radiolabeled antibody localized in the tumor was low compared with that localized in the blood and other organs, particularly the liver, thus limiting the clinical utility of present methods.

Radioimmunotherapy

Ettinger et al (17) used ^{131}I-labeled antiferritin IgG and ^{131}I-labeled anti-CEA IgG as part of an integrated treatment program for primary hepatic malignancies (cholangiocarcinoma and hepatoma). Data have been published on 18 patients who received 37-157 mCi of radiolabeled whole immunoglobulin antibody. An objective antitumor effect was noted in six of nine patients evaluable for response. The treatment scheme involved the use of external irradiation, chemotherapy, and radiolabeled antibody in the same patients. The use of combined therapy makes it difficult to assess the toxicity and therapeutic benefits of the radiolabeled antibody as distinct from the other modalities. Nonetheless, the results are encouraging. The problem of antitumor dosimetry with radiolabeled murine fragments has been studied by Leichner et al (18) for the case of ^{131}I-labeled antiferritin antibody in hepatoma. The kinetics of uptake and clearance of whole IgG into tumor and liver is slow, with half-lives in the order of several days. Thus, radiation dose is delivered over a long period of time, with a maximum dose rate to tumor of about 6 rads/hour for whole antiferritin IgG. The dose to tumor is about 1500-2200 rads for millicurie doses ranging from 97 to 157 mCi of the whole-immunoglobulin preparation. Marrow is estimated to receive about one-tenth of the radiation dose to tumor and is thus the dose-limiting organ.

Studies With Monoclonal Antibody and Immune Fragments

Perhaps the most important and revolutionary technical innovation in the field of immunology has been the development of the hybridoma technology described by Kohler and Milstein (19). This has facilitated development of highly specific and reproducible antibodies of a given class, which can be produced in essentially unlimited quantities. The application of this technology for the diagnosis and treatment of cancer is being actively pursued. In vivo experiments in animal models have shown excellent localization in transplanted xenografts of human tumors (2,20,21). Therapy with stable murine monoclonal antibodies (MOAB) in animal models has inhibited growth of solid tumor xenografts (22,23) and leukemia (24,25). Several groups have reported their initial experience in humans with stable MOAB therapy of cutaneous T-cell lymphoma (26), T-cell leukemia (27), and acute lymphoblastic leukemia (28). Trials with conjugated drugs and toxins have been successful in in vitro models (29) and absorbed polysera carrying chlorambucil have been used in vivo in animals and humans with melanoma (30). From the standpoint of radioimmunodetection and radioimmunotherapy, MOAB offer significant advantages over polysera, because of the reproducibility of the antibodies and their availability in large quantities. This permits careful characterization of the MOAB and allows for selecting among a variety of antibody types to obtain the optimal antibody as carrier for in vivo localization of radioactivity. For example, monoclonal antibodies are available to a variety of tumor-associated antigens, and in some cases it is possible to select from among a family of antibodies directed against the same antigen, with respect to (*a*) subclass of IgG, (*b*) epitope that is recognized by the antibody, (*c*) affinity of binding of antibody to antigen, and (*d*) cross-reactivity with normal tissue antigens. In addition, it is also possible to employ whole IgG or immune fragments, ie, Fab, Fab′, or $F(ab')_2$. The number of possible combinations is very large, and at present it is not possible to state definitely the total range of characteristics which is ideal for an antitumor antibody for in vivo localization. In general, we have found that high-affinity antibodies ($k = 10^{10}$ L/M), with low normal tissue binding (no detectable cross-reactivity with normal blood cells), are the most useful for in vivo localization studies. A number of investigators have used monoclonal antibodies to successfully image human tumors (31-34), particularly anti-CEA antibodies for colon.

Studies in Human Melanoma

We are evaluating a new diagnostic and therapeutic strategy utilizing MOAB and their fragments for diagnosis and treatment of malignant melanoma. Melanoma is an aggressive tumor, usually arising in the skin; once it metastasizes the response rate to conventional treatment (chemotherapy, radiation therapy, or immunotherapy with BCG) is poor. We have targeted two melanoma-associated antigens. The antigen best characterized is p97, a 97,000 molecular weight glycoprotein on the cell surface, which is present in concentrations of up to 400,000 molecules/cell. It is expressed at very low concentrations in most normal adult tissues but at high levels in > 50% of melanomas and in fetal colon, thus characterizing it as an oncofetal antigen (35-37). Sequencing of the amino acid structure shows 60% homology with serotransferrin and lactotransferrin (38). Several MOAB have been developed against different epitopes (35). The second antigen system we have targeted is a proteoglycan on the cell surface, with weights of 250 and 400 kd described as high-molecular-weight antigen; a MOAB, 48.7 (IgG1), has been developed (39).

Imaging Results

Our initial work utilizing whole IgG (anti-p97) for imaging patients with metastatic melanoma showed localization of 88% of the lesions > 1.5 cm in diameter, and comparison with simultaneous administration of ^{125}I nonspecific antibody in tumor biopsies documented specific uptakes (32). The two major problems identified were the prolonged circulation in blood pool with low-target to non-target ratios and the early appearance of human antimouse response.

We have utilized anti-p97 Fabs for diagnostic imaging.[5] Thirty-three patients were studied and 20 had positive scans. The 13 negative scans (no tumor visualization) are explained as follows: ten patients had disease < 1.5 cm, which is at the limit of resolution of the gamma camera; two patients had low p97 levels; and in one patient the iodination was a technical failure, with only 3% of the product being immunoreactive. We have studied ten patients with ^{131}I-Fab 48.7, with tumor localization in eight. The two patients whose tumor had no visualization had minimal disease (< 1 cm).[6] Based on biodistribution data obtained from localization studies with anti-p97, dosimetry estimates were made; for every 100 mCi of ^{131}I-Fab administered, the tumor received 1040 rads, the liver received 325 rads, and the marrow received 30 rads.[5]

In this report we include our experience with therapy in ten patients, seven of whom have been previously reported.[5]

MATERIALS AND METHODS

Fab Production

Three different preparations were utilized for treatment. Two Fabs were specific for p97 (35): antibody 8.2 (IgG1) and antibody 96.5 (IgG2a). The other antibody, 48.7, was directed against a high-molecular-weight antigen (39). The antibodies were purified from the ascites fluid of mice bearing hybridoma ascites tumors by affinity chromatography on protein A-Sepharose (35). Fab fragments were prepared by papain digestion (36).

---

[5] Larson SM, Carrasquillo JA, Krohn KA, et al. Localization of I-131 labeled p97 specific Fab fragments in human melanoma as a basis for therapy. *Journal of Clinical Investigations*, in press.

[6] Larson SM, Carrasquillo JA, Krohn KA, et al. Radiotherapy using an I-131 labelled Murine Fab against a high molecular weight antigen of melanoma. Manuscript in preparation.

Radiolabeling

All MOAB Fabs were radioiodinated by the chloramine-T (CT) method. ^{131}I was obtained at high specific activity as protein labeling grade in 0.1 M NaOH (New England Nuclear, North Billerica, MA). Labeling was performed remotely in a hot cell (42), with ratios of 25 μg of CT/mg of Fab.

The final preparation was in 0.05 M phosphate-buffered saline suitable for injection. The percent protein-bound iodine was determined by using cellulose acetate thin-layer electrophoresis (typically $> 95\%$); the products were determined to be pyrogen-free prior to injection (Limulus Amoebocyte Lysate, Associates of Cape Cod, Woods Hole, MA) and sterility testing was performed.

Immunohistologic Staining

We used an immunohistochemical technique for antigen localization in tissue sections, ie, the Sternberger technique (40), which was modified by us (41).

Assay for Human Antimouse Antibody (HAMA)

A test sample of the patients' serum (10 μl) was incubated with 10 μl of ^{125}I-labeled mouse IgG (10^4 cpm) for 30 minutes at 20°C. *Staphylococcus aureus* (10 mg) was added in 1 ml of 20 mM Tris-HCl buffer, pH 8.0, containing 100 mM NaCl, 1 mM EDTA, and 0.5% Nonidet P40 (TNEN). After a further 5-minute incubation, the bacteria were separated by centrifugation and washed three times with 10 ml of TNEN and counted for ^{125}I. A comparison was made between the test sample and a control serum that did not contain HAMA. A positive test was considered to be a value twice the control.

Human Studies

All patients were studied under IND BB 1609 after approval by all appropriate institutional review committees and the patients' informed consent. All patients were noncandidates for curative resection, and they were preselected for high melanoma-associated antigen levels in their tumor. They then underwent one or more localization studies with the selected Fab, anti-p97 or 48.7, to determine the degree of tumor uptake. The patients with avid tumor uptake were then selected for therapy.

Prior to iv injection, all patients underwent intradermal skin tests with the unlabeled Fab (20 μg). Thyroid uptake was blocked with ten drops of a saturated solution of potassium iodide (SSKI) 24 hours prior to treatment and twice per day for 14 days after injection. This dose of SSKI will reduce the thyroid uptake of $Na^{131}I$ to about 1% of the dose (42) and is administered since some free iodine is liberated from the radiolabeled Fab. Baseline laboratory tests were obtained before injection and were repeated every 2 weeks for 6 weeks or until stable. These tests included hemoglobin, wbc counts, serum creatinine, bilirubin, SGOT, alkaline phosphatase, and urinalysis. The antibody was then infused over a 1-hour period with a Harvard infusion pump, and blood samples were drawn serially for biodistribution measurements. The patients were scanned by a gamma camera with a high-energy parallel hole collimator when activity was approximately < 20 mCi and at 1 week.

RESULTS

We have performed localization studies with anti-p97 in 40 patients and with 48.7 in ten patients. We have selected seven patients for treatment with ^{131}I anti-p97 Fab and three for treatment with 48.7 on the basis of avid tumor uptake of the ^{131}I antimelanoma Fab on the diagnostic study. Figure 1 shows a ^{131}I-labeled 48.7 Fab diagnostic scan at 48 hours on a patient with metastatic disease to the liver as seen on his liver scan. The uptake measurements were semiquantitative approximations based on region of interest data (generated from digitized gamma camera images using a dedicated nuclear medicine computer, Technicare VIP 450, Solon, OH) obtained over tumor versus nontarget tissue.

Table 1 is a summary of data on the study population. The mean age was 46.2 years (range, 31-65). There were two females and eight males. Localization and therapy studies targeting p97 were performed with either Fab 8.2 or 96.5, and no significant differences in biodistribution were observed. There was a clear difference in loss of immunoreactivity at higher specific activities greater for 8.2 than for 96.5.[7] The seven patients treated with ^{131}I anti-p97 Fab received individual doses of 30-342 mCi and cumulative doses of 132-861 mCi. The first three patients received the ^{131}I treatment carried by 4 mg of Fab. All subsequent Fab administration consisted of 10 mg of Fab, with the exception of the 342-mCi dose given Patient 7, which was carried on 20 mg of Fab. The larger amounts of Fab were necessary when escalating the dose to maintain good immunoreactivity.

Seven patients were not evaluable for therapeutic re-

---

[7] Ferens JM, Beaumier PL, Link JM, et al. Preparation of radioiodinated murine monoclonal antibody fragments at high activity levels. Abstract NUCL 104, 185th American Chemical Society National Meeting, Seattle, Mar 1983.

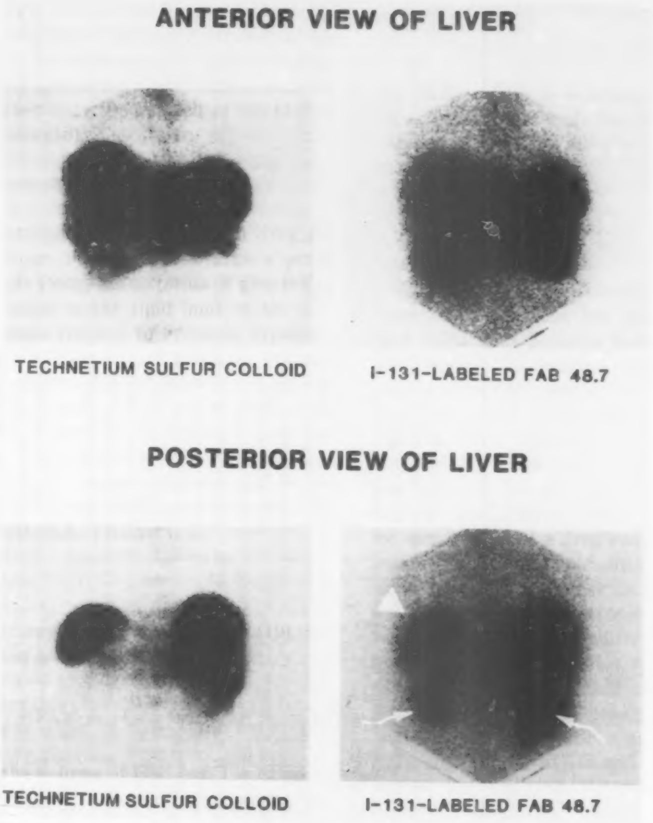


FIGURE 1.—Upper left panel shows a conventional liver-spleen scan, with ^{99m}Tc-sulfur colloid (TcSC) demonstrating multiple focal defects secondary to extensive metastatic disease. In the right upper panel is a corresponding anterior view of the liver at 48 hrs after injection of ^{131}I-labeled 48.7. The normal distribution of 48.7 does not demonstrate liver uptake, but in this patient diffuse uptake in liver metastases was seen. Lower left panel shows a posterior TcSC liver-spleen scan view demonstrating liver metastases. The right lower panel shows the corresponding ^{131}I-labeled 48.7 image with diffuse uptake in the liver metastases. The spleen (white arrowhead) is normally visualized and probably indicates some cross-reacting antigen. The kidneys (white curved arrows) are a route of excretion.

sponse either because of the low dose administered or because of their advanced disease and short survival (median, 24 days; range, 14-150) after treatment (table 1). These patients received a limited dose which at best would have delivered an estimated 2080 rads to tumor. Only three patients were evaluable because of their length of follow-up (mean, > 218 days; range, 90 to > 394) and delivered dose (374-861 mCi), with an estimated 3800-8500 rads to tumor. Their course is described below. Patient 5 had rapidly enlarging liver metastases.[5] A scan performed 1 week after his first dose showed good concentration in his tumor (fig 2B). Figure 2A is a TcSC liver-spleen scan in the same projection for comparison. Transmission CT scan re-evaluation after two doses (329 mCi) showed no significant effect on his tumor growth as seen in figure 3. A third dose of 174 mCi was ineffective because of in vivo development of HAMA with neutralization of Fab. He developed progressive disease and died 90 days after the first treatment dose.

Patient 7 received three doses totaling 519 mCi, carried on total doses of 30 mg of Fab; his tumor response is shown in figure 4. There was stabilization of his previously rapid growing liver metastasis for 3 months, which subsequently continued to increase in volume as determined by transmission CT measurements. A

TABLE 1.—Summary of data on study population

Patient No.	Date	Fab	mg	^{131}I mCi	Clinical history	Follow-up*	Toxicity
1	9/29/81†	8.2	0.8	4	57-yr-old female with ocular melanoma resected 7 yrs prior to study; liver and subcutaneous metastases were treated 2 yrs before without success (cyclophosphamide, dacarbazine); no therapy for 6 wks prior to Fab treatment	Died at 24 days	HAMA, positive; urticaria
	11/10/81‡	1.4	1.32	4			
	12/9/81†	8.2	0.76	5			
	3/23/82	8.2	4.0	34			
	4/21/82	96.5	4.0	90			
2	1/19/82†	8.2	0.86	3	52-yr-old male with resected primary left nares; 23 yrs prior to study, 18 yrs after he developed cervical nodes and subcutaneous lesions; treated with actinomycin and vincristine with good response, but later developed lung and subsequently brain metastases	Died at 33 days	HAMA, negative
	3/2/82†	96.5	0.4	4			
	3/9/82†	96.5/8.2	0.8	3			
	3/24/82	8.2	4.0	135			
3	1/19/82†	8.2	0.82	3	37-yr-old male with resection of primary in right forearm with wide excision and axillary dissection (+ nodes); treated with regional perfusion (melphalan) 9 mos prior to Fab; 5 mos before developed urinary and pubic metastases; 1 mo before 1st Fab treatment developed brain, lung, and massive peritoneal involvement	Died at 14 days	HAMA, negative
	3/2/82†	8.2	0.82	3			
	3/9/82†	96.5/8.2	0.82	3			
	4/1/82	8.2	4.0	125			
4	5/12/82†	8.2	5.0	10	60-yr-old male with ocular melanoma diagnosed and enucleated 28 mos before treatment; developed metastases in liver, lung, adrenal, pancreas, kidney, bone marrow, and lymph nodes	Died at 26 days	HAMA, NA§
	6/2/82	8.2	10	165			
5	6/2/82†	8.2	5	8	34-yr-old male with primary on right shoulder; had excision with axillary dissection; at time of study had extensive liver metastases and subcutaneous disease; he neutralized the last therapy dose injected because of HAMA	Died at 100 days	HAMA, positive; rash, maculopapular; 50% drop in leukocyte and platelet counts
	6/9/82	8.2	10	192			
	6/30/82	8.2	10	137			
	7/14/82	8.2	10	174			
6	7/21/82†	96.5	5	16	31-yr-old male with primary right shoulder treated with excision and axillary disection (- nodes); immunotherapy with BCG given for 4 yrs; 7 mos before study developed lung metastases and failed treatment with interferon, vinblastine, and bleomycin; last chemotherapy was 5 mos before Fab; at time of treatment he had brachial plexus involvement, lung, brain, and liver	Died at 20 days	HAMA, NA
	8/4/82	96.5	10	169			

7	6/23/82†	8.2	5	9	53-yr-old male with ocular melanoma arising in ciliary body and extending into sclera; underwent enucleation and was free of disease until Oct 1981 (3 yrs) when he developed subcutaneous nodules in the scalp which were resected; disease recurred in June 1982 with liver, renal, and subcutaneous metastases; no chemotherapy or radiation therapy	Alive > 394 days	HAMA, positive
	7/7/82	8.2	10	159			
	7/29/82	8.2	10	180			
	8/18/82	96.5	10	180			
	10/28/82	96.5	20	342			
8	2/2/83†	48.7	2.0	5	40-yr-old female with right elbow melanoma resected and axillary dissection performed 4 yrs before Fab study; 2 yrs later found with liver metastasis, failed dacarbazine, but had good response to lomustine which was discontinued because of marrow toxicity; received radiation therapy for pelvic metastases; at the time of treatment had liver, lung, lymph, and bone disease	Died at 150 days	HAMA, negative; marrow toxicity, mild; erythema and blistering at extravasation site
	2/16/83	48.7	10	142			
9	2/23/83†	48.7	3.5	8	33-yr-old male with wide excision of primary melanoma from low back 2 yrs prior to study; 1 mo before Fab treatment he developed inguinal adenopathy and CT evidence of iliac nodes, with biopsy-documented melanoma; no prior chemotherapy; transmission CT documented response but high HAMA titers did not permit subsequent treatments	Died at 150 days	HAMA, positive
	3/2/83†	48.7	10	19			
	3/9/83†	96.5	10	21			
	3/16/83	48.7	10	181			
	3/30/83	48.7	10	193			
10	4/13/83†	96.5¶	1	5	65-yr-old male with wide excision of primary from left scapular region 4 yrs before Fab treatment; developed metastases to right lung and underwent lobectomy; 2 mos later found to have extensive involvement of liver and lung; no prior radiation therapy or chemotherapy at entry in Fab study	Died at 15 days	HAMA, positive
	4/27/83†	48.7	5	8			
	5/4/83	48.7	10	202			

---

* Follow-up since 1st therapy.

†Localization studies (< 30 mCi).

‡Nonspecific antibody.

§NA = not available.

¶^{111}In-IgG.

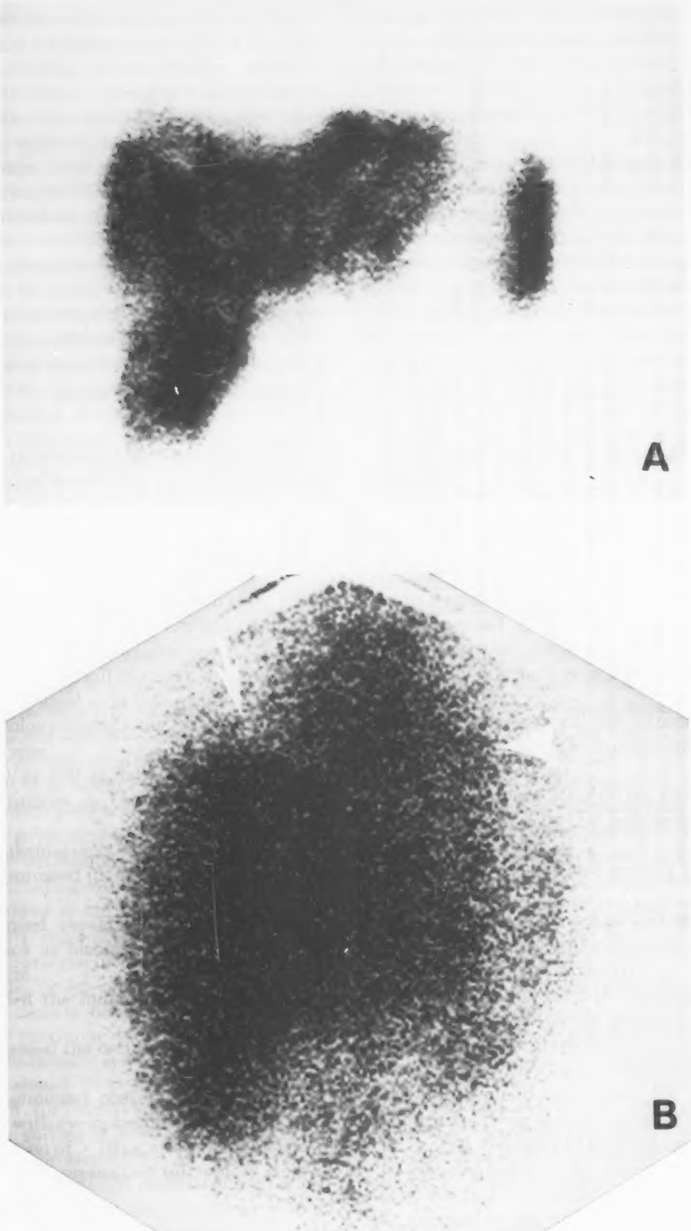


FIGURE 2.—A. Anterior TcSC liver scan shows multiple metastatic lesions, the largest of which is in the dome of the liver (white arrow). B. Anterior view of the liver 1 wk after administration of 192 mCi of ^{131}I anti-p97 showing diffuse uptake of the tracer in multiple metastatic lesions. Blood pool activity is also visualized (white arrowhead).

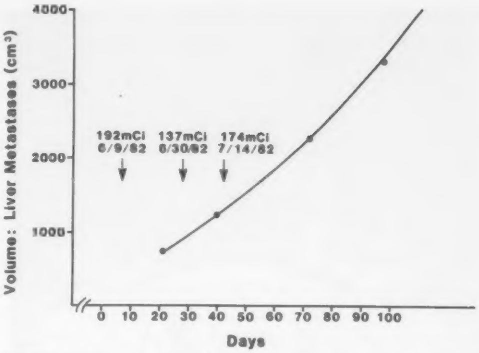


FIGURE 3.—Tumor volume determinations were measured from a sequence of transmission CT slices (45), prior to and at different times after initial evaluation with anti-p97 Fab. The dates of treatment and millicuries used are indicated with arrows and the solid dots indicate the days of serial transmission CT re-evaluation. In this case tumor growth continued despite therapy.

fourth dose with 342 mCi of ^{131}I anti-p97 was administered and stabilization for < 2 months was seen with subsequent rapid tumor growth. No further treatments were given because he had developed severe bone marrow toxicity and also had high titers of HAMA.

The third evaluable patient (No. 9) had metastatic disease to inguinal and pelvic nodes. He received two treatments totaling 374 mCi within a 2-week interval. A comparison of pre- and 6-week post-treatment transmission CT showed a > 50% reduction of disease (fig 5) and disappearance of some nodes with no new lesions visualized. A third treatment dose was withheld because of high levels of HAMA and very positive skin test. Tumor growth was again noted 3 months later, with new lung lesions and enlargement of pelvic nodes.

Toxicity has been limited. No significant changes in pre- and post-treatment liver function or renal function test were observed. Although no abnormalities in hemoglobin, neutrophil, or platelet counts were seen in the diagnostic studies, patients receiving therapy with > 500 mCi of ^{131}I Fab showed a 50% drop in neutrophil and platelet counts, with nadirs at 3-4 weeks and gradual improvement thereafter. In one patient (No. 8) a 50% drop in platelet and a 32% drop in wbc counts occurred after only 142 mCi. This patient had had prior intensive chemotherapy as well as radiation to her

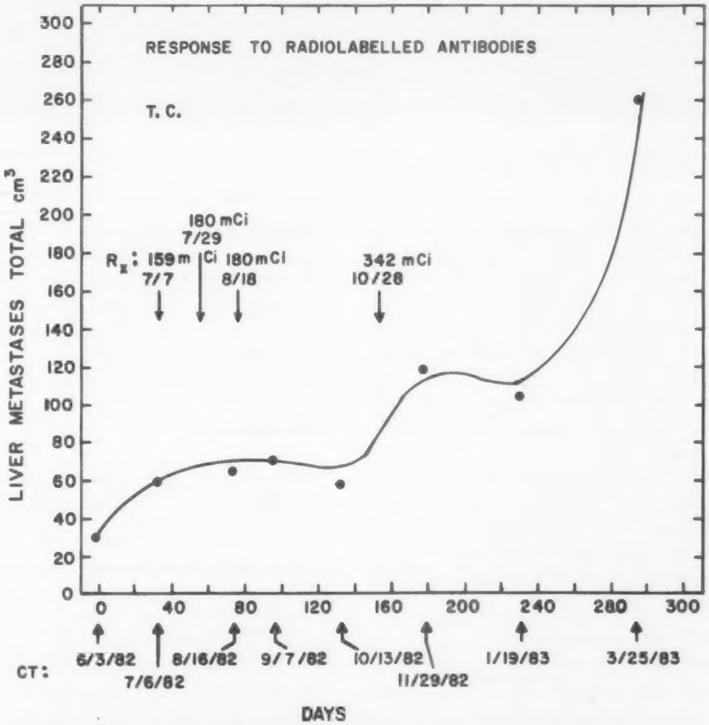


FIGURE 4.—Tumor volume determinations in Patient 7 during his course of treatment are shown. Days of treatment and millicuries received are indicated. Arrows below the abscissa represent the day of serial transmission CT (*see* text).

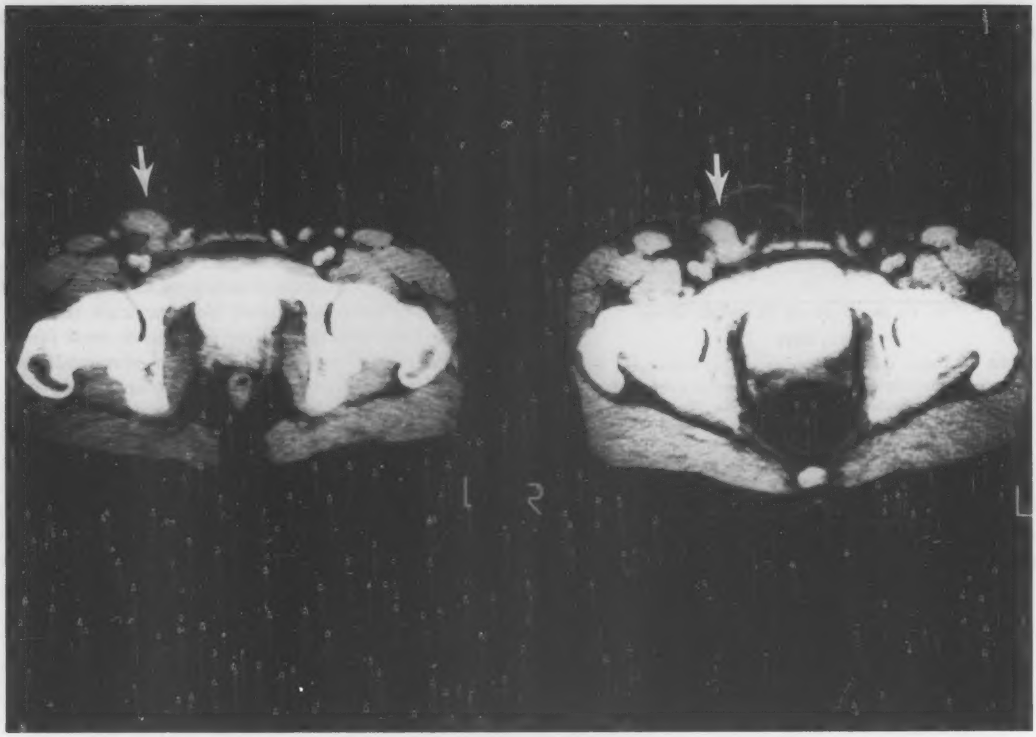


FIGURE 5.—Transmission CT obtained for Patient 9, prior to treatment (left panel) and 6 wks after treatment. Corresponding sections through the inguinal region showed a > 50% reduction in an inguinal node (white arrow).

right proximal femur and had depressed platelet and leukocyte counts (130,000 and 3200-4000/mm^3, respectively) at entry in the study. One patient (No. 7) had severe marrow toxicity; he received 342 mCi of Fab at a single dose after prior cumulative therapy with approximately 519 mCi, and 4 weeks after treatment his platelet count was 17,000/mm^3 and his neutrophil count was 700/mm^3. He was given 7 units of platelets prophylactically and gradually improved after treatment without any complication.

A total of 86 radiolabeled Fab injections have been administered (five patients had received IgG previously); 36 of these injections were administered to the ten therapy patients (table 2). Seventeen patients receiving Fab injections were tested for HAMA (table 3) at varying times during their studies. Three of these patients developed HAMA after one injection, two after three injections, and three after four injections: eight of 17 patients (47%). Nine patients did not develop HAMA. Seven of these patients had multiple injections. In contrast, seven of nine patients who received a single dose of whole IgG developed HAMA (78%).

TABLE 2.—Antibody administration

	No. of administrations				
	1	2	3	4	5
Total No. of patients	20	10	5	4	4
No. of therapy patients	0	3	1	3	3

DISCUSSION

We are evaluating a new diagnostic and therapeutic strategy utilizing ^{131}I-labeled antimelanoma Fab direct-

TABLE 3.—HAMA response to Fab administration*

No. of previous injections	HAMA positive	HAMA negative
1	3	2
2	0	5
3	2	2
4	3	0

* All studies performed 14 days after Fab administration.

ed against melanoma-associated antigens. Our preliminary imaging and therapy results are encouraging and have revealed several problems that can now be addressed to improve the diagnostic and therapeutic potential. These problems include (*a*) development of HAMA, (*b*) heterogeneity of tumor localization, (*c*) liver uptake of radioactivity, (*d*) complexing of antibody by circulating antigen, and (*e*) in vivo deiodination of antibody.

One of the major problems that we have encountered is the rapid development of HAMA. This has been a frequent finding in patients who receive single doses of whole IgG (78%) and has prompted us to utilize Fab fragments. While Fab appears to be less immunogenic than whole IgG, most patients developed an immune response after multiple injections, although infrequently we have seen low titers even after single injections. The clinical manifestations associated with development of immunity were relatively mild allergic reactions. From the standpoint of in vivo localization, the more important consequence of high HAMA titers has been major alterations in biodistribution, with rapid clearing into the liver, and marked reduction of tumor uptake, making further diagnostic or therapeutic administrations fruitless.

Work with human monoclonal antibody may be helpful in eliminating the host immune response and thus permitting repeated injections of either conjugated, stable, or radiolabeled MOAB. Other methods to create tolerance such as simultaneous immune suppression with chemotherapeutic agents may be necessary. We will be exploring the feasibility of utilizing plasmapheresis or affinity columns to eliminate HAMA once formed. A similar approach has been used successfully in cases of ABO incompatibility (43).

Antigen heterogeneity for the tumor antigens in vivo is obvious in multiple biopsy sections from patients. Autopsy data on two of our patients have shown significant heterogeneity of radioactivity concentration from tumor site to site and even within one tumor site (data not shown). This is probably the explanation of the variability of in vivo tumor localization seen with the whole-body imaging.

The use of "cocktails" of MOAB antibodies of known specificity could significantly enhance tumor uptake by permitting targeting of several tumor-associated antigens simultaneously. The biodistribution of Fab 96.5 and 8.2 was identical, which was not surprising since they are both directed at different epitopes on p97 (37). For anti-p97 the most salient biodistribution feature was significant concentration in the liver,[5] with the disadvantage that localization of liver metastases was harder to visualize because of the high background, and the radiation dose to the liver was high. In contrast, antibody 48.7 does not concentrate in the normal liver but does have some concentration in the spleen. These differences in biodistribution are likely secondary to cross-reacting antigens. The in vivo biodistribution and dosimetry of different MOAB directed at other tumor-associated antigens are likely to show different patterns of distribution depending on cross-reactivity. It will be important to recognize the idiosyncrasy of each preparation for proper interpretation of diagnostic studies.

The circulating levels of p97 in plasma of patients with melanoma are 8.36 ± 8.04 ng/ml (mean ± SD, J. Reynolds, personal communication). In our early studies, when low doses of IgG (50 μg) were administered, most of the activity was complexed to antigen (data not shown) and for this reason we increased the dose up to 10 mg of Fab/patient. This is likely to be a significant problem in the cases of antigens such as CEA, which tend to be shed in the blood. In vitro data with a CEA model system have demonstrated decreased tumor uptake and increased liver uptake as levels of circulating CEA are increased (44). In the future we may have the option of selecting tumor antigens that are not significantly shed into plasma or, alternatively, it may be necessary to pretreat with stable antibody to clear the circulating antigen, then to administer the radiolabeled antibody.

After administering ^{131}I-labeled IgG or Fab to patients, in vivo deiodination does occur, particularly in the liver and kidney. For the antimelanoma preparation which we have used, we estimate that about 20%–30%/day of the contained radioactivity is deiodinated, since most of the excreted radioactivity is cleared as iodide ^{131}I, and about 36% of the total administered is excreted/day. More stable radiolabeled preparations are being sought which do not break down as rapidly in vivo (^{111}-In-chelated antibodies), or more stable iodine derivatives.

No major organ toxicity attributable to the radiolabeled Fab was observed. At the diagnostic level no significant changes in hematocrit, neutrophil count, or platelet count have been observed. The therapy studies were relatively well-tolerated, with marrow toxicity in general occurring at levels of approximately 500 mCi and severe toxicity occurring in the only patient who received levels beyond this range, suggesting that cumulative doses of up to 800 mCi may be administered. However, further experience is necessary before determining the dose level at which severe toxicity will be seen.

Due to the nature of our study our patient population was biased toward patients with far-advanced disease and extremely poor prognosis; nonetheless, we are encouraged with the response observed in two of our three evaluable patients and plan further studies to optimize the radiation dose delivery for treatment of metastatic melanoma.

REFERENCES

1. GOLD P, and FREEDMAN SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med 122:467-481, 1965.
2. COLCHER D, ZALUTSKY M, KAPLAN W, ET AL. Radiolocalization of human mammary tumors in athymic mice by a monoclonal antibody. Cancer Res 43:736-742, 1983.
3. ABELEV GI. Alpha-fetoprotein in oncogenesis and its association with malignant tumors. Adv Cancer Res 14:295-358, 1971.
4. HELLSTROM KE, and HELLSTROM I. Antigens in human melanomas detected by using monoclonal antibodies as probes. *In* Melanoma Antigens and Antibodies. New York, Plenum Press, 1982, pp 187-206.
5. LEE LH. The biosynthesis of hormone by nonendocrine tumours—a review. J Endocrinol 67:143-175, 1975.
6. GOLDENBERG DM, DELAND F, KIM EE, ET AL. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. N Engl J Med 298:1384-1388, 1978.
7. PRESSMAN D. Radiolabeled antibodies. Ann NY Acad Sci 69:644-650, 1957.
8. MCINTIRE KR, VOGEL CL, PRINCLER GL, ET AL. Serum alpha-fetoprotein as a biochemical marker for hepatocellular carcinoma. Cancer Res 32:1941-1946, 1972.
9. MACH JP, CARREL S, FORNI M, ET AL. Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma. N Engl J Med 303:5-10, 1980.
10. HINE KR, BRADWELL AR, REEDER TA, ET AL. Radioimmunodetection of gastrointestinal neoplasms with antibodies to carcinoembryonic antigen. Cancer Res 40:2993-2996, 1980.
11. GOLDENBERG DM, KIM EE, DELAND FH, ET AL. Radioimmunodetection of cancer using radioactive antibodies to human chorionic gonadotropin. Science 208:1284-1286, 1980.
12. GOLDENBERG DM, KIM E, DELAND FH, ET AL. Clinical studies on the radioimmunodetection of tumors containing alpha-fetoprotein. Cancer 45:2500-2505, 1980.
13. KIM EE, DELAND FH, and NELSON MO. Radioimmunodetection of cancer with radiolabeled antibodies to alpha-fetoprotein. Cancer Res 40:3008-3012, 1980.
14. BELITSKY P, GHOSE T, AQUINO J, ET AL. Radionuclide imaging of metastases from renal-cell carcinoma by ^{131}I-labeled antitumor antibody. Radiology 126:515-517, 1978.
15. ORDER SE, BLOOMER WB, JONES AG, ET AL. Radionuclide immunoglobulin lymphangiography: a case report. Cancer 35:1487-1492, 1975.
16. PRIMUS FJ, and GOLDENBERG DM. Immunological considerations in the use of goat antibodies to carcinoembryonic antigen for the radioimmunodetection of cancer. Cancer Res 40:2979-2983, 1980.
17. ETTINGER DS, ORDER SE, WHARAM MD, ET AL. Phase I-II study of isotopic immunoglobulin therapy for primary liver cancer. Cancer Treat Rep 66:289-297, 1982.
18. LEICHNER PK, KLEIN JL, GARRISON JB, ET AL. Dosimetry of 131-labeled antiferritin in hepatoma: a model for radioimmunoglobulin dosimetry. Int J Radiat Oncol Biol Phys 7:323-333, 1981
19. KOHLER G, and MILSTEIN C. Continuous cultures of fused cells secreting antibody of proven defined specificity. Nature 256: 495-497, 1975.
20. MOSHAKIS V, MCILHINNEY RAJ, RAGHAVAN D, ET AL. Localization of human tumor xenografts after intravenous administration of radiolabeled monoclonal antibodies. Br J Cancer 44:91-99, 1981.
21. HOUSTON LL, NOWINSKI RC, and BERNSTEIN ID. Specific in vivo localization of monoclonal antibodies directed against the Thy 1.1 antigen. J Immunol 125:837-843, 1980.
22. HERLYN DM, STEPLEWSKI Z, HERLYN MF, ET AL. Inhibition of growth of colorectal carcinoma in nude mice by monoclonal antibody. Cancer Res 40:717-721, 1980.
23. ______. Monoclonal antibodies in cell-mediated cytotoxicity against human melanoma and colorectal carcinoma. Eur J Immunol 9:657-659, 1979.
24. SCHEINBERG DA, and STRAND M. Leukemic cell targeting and therapy by monoclonal antibody in a mouse model system. Cancer Res 42:44-49, 1982.
25. BERNSTEIN ID, TAM MR, and NOWINSKI RC. Mouse leukemia: therapy with monoclonal antibodies against a thymus differentiation antigen. Science 207:68-71, 1980.
26. MILLER RA, and LEVY R. Response of cutaneous T cell lymphoma to therapy with hybridoma monoclonal antibody. Lancet 2:226-230, 1981.
27. MILLER RA, MALONEY DG, MCKILLOP J, ET AL. In vivo effects of murine hybridoma monoclonal antibody in a patient with T cell leukemia. Blood 58:78-86, 1981.
28. RITZ J, PESANDO JM, SALLAN SE, ET AL. Serotherapy of acute lymphoblastic leukemia with monoclonal antibody. Blood 58:141-152, 1981.
29. CASELLAS P, BROWN JP, GROS P, ET AL. Human melanoma cells can be killed in vitro by an immunotoxin specific for melanoma associated antigen p97. Int J Cancer 30:437-443, 1982.
30. GHOSE T, NORVELL ST, GUCLU A, ET AL. Immunochemotherapy of cancer with chlorambucil-carrying antibody. Br Med J3:495-499, 1972.
31. LARSON SM, CARRASQUILLO JA, KROHN KA, ET AL. Diagnostic imaging of malignant melanoma with radiolabeled antitumor antibodies. JAMA 249:811-812, 1983.
32. LARSON SM, BROWN JP, WRIGHT PW, ET AL. Imaging of melanoma with I-131-labeled monoclonal antibodies. J Nucl Med 24:123-129, 1983.
33. HALPERN SE, DILLMAN RO, HAGAN PL, ET AL. The clinical evaluation of 111-Indium labeled monoclonal anti-melanoma antibodies (111-In-anti mel) for tumor scanning. J Nucl Med 24:P15, 1983.
34. MACH JP, BUCHEGGER F, FORNI M, ET AL. Use of radiolabelled monoclonal anti-CEA antibodies for the detection of human carcinomas by external photoscanning and tomoscintigraphy. Immunol Today 2:239-249, 1981.
35. BROWN JP, NISHIYAMAU K, HELLSTROM I, ET AL. Structural characterization of human melanoma-associated antigen p97 using monoclonal antibodies. J Immunol 127:539-546, 1981.
36. BROWN JP, WOODBURY RG, HART CE, ET AL. Quantitative analysis of melanoma-associated antigen p97 in normal and neoplastic tissues. Proc Natl Acad Sci USA 78:539-543, 1981.
37. WOODBURY RJ, BROWN JP, YEH MY, ET AL. Identification of a cell surface protein, p97, in human melanomas and certain other neoplasms. Proc Natl Acad Sci USA 77:2183-2186, 1980.
38. BROWN JP, HEWICK RM, HELLSTROM I, ET AL. Human melanoma-associated antigen is structurally and functionally related to transferrin. Nature 296:171-173, 1982.
39. HELLSTROM I, GARRIGUES J, CABASCO L, ET AL. Studies of a high molecular weight human melanoma-associated antigen. J Immunol 130:1467-1472.
40. STERNBERGER LA. The unlabelled antibody peroxidase-antiperoxidase (PAP) method. *In* Immunocytochemistry. New York, John Wiley & Sons, Inc, 1979, pp 104-169.
41. GARRIGUES HJ, TILGEN W, HELLSTROM I, ET AL. Detection of a human melanoma-associated antigen, p97, in histological sections of primary human melanomas. Int J Cancer 29:511-515, 1982.
42. STERNTHAL E, LIPWORTH L, STANLEY B, ET AL. Suppression of thyroid radioiodine uptake by various doses of stable iodide. N Engl J Med 303:1083-1088, 1980.
43. BENSINGER WI. Plasma exchange and immunoadsorption for removal of antibodies prior to ABO incompatible bone marrow transplant. Artif Organ 5:254-258, 1981.
44. HAGAN PL, HALPERN SE, CHEN A, ET AL. Effects of tumor size, carcinoembryonic antigen (CEA) production and secretion on the distribution of ^{111}-In and ^{125}-I monoclonal antitumor antibodies (MOAB) in animal model. J Nucl Med 24:P77, 1983.
45. MOSS AA, FRIEDMAN MA, and BRITO AC. Determination of liver, kidney and spleen volumes by computed tomography: an experimental study in dogs. J Comput Assist Tomogr 5:12-14, 1981.

Cancer Treatment Reports 1959–1983: Background Review and Tabular Compilation of Most Cited Articles

Eleanor M. Sloane,* Jean Griffin Baum, Susan Molloy Hubbard, and Robert E. Wittes[1]

Cancer Treatment Reports (CTR), first printed in January of 1959 as *Cancer Chemotherapy Reports*, "was established to provide a vehicle for the prompt and informal exchange of technical information among participants in the Cancer Chemotherapy National Service Center (CCNSC) program and other interested investigators." From 1959 to 1968 the journal was issued 6–10 times a year, depending on the number of manuscripts submitted and accepted for publication. In 1968 the journal was approved as a periodical by the Office of Management and Budget and was expanded to three distinct parts:

Part 1:	Original research, both experimental and clinical;
Part 2:	Comprehensive, chemotherapy studies involving tabular material; and
Part 3:	Program information including treatment protocols, clinical brochures, toxicology reports, and review articles.

In 1959 when the journal was first issued to the public, the Chairman of the Editorial Board was Dr. Jonathan Hartwell and the Editor-in-Chief was Dr. Stuart M. Sessoms. These two individuals together shepherded the journal until 1962 when the CCNSC was established with Dr. Kenneth Endicott as Director. At that time, Dr. Hartwell became the Scientific Editor of *CTR* and Dr. Sessoms became a member of the journal's Advisory Board. In 1966, Dr. Jerome B. Block, Dr. John P. Glynn, and Dr. J. A. R. Mead governed the Editorial Board, with Dr. Vincent T. DeVita joining them in 1970. In 1972 an official "Editor-in-Chief" position was established and Dr. Mead became the first Editor-in-Chief. He was succeeded by Dr. Bruce A. Chabner (July 1976), Dr. John S. Macdonald (August 1979), and Dr. Robert E. Wittes (April 1983).

In January 1976, the scope of *CTR* was broadened from a drug development publication to a journal that considers original manuscripts from all research areas encompassed by the programs of the Division of Cancer Treatment (DCT). The journal now accepts unsolicited and previously unpublished manuscripts in all areas of treatment-related research including medicinal chemistry, chemotherapy, pharmacology, cell biology, cell kinetics, pediatric oncology, medical oncology, surgery, radiation therapy, radiation biology, and immunotherapy. It was also decided that, when appropriate, the Editorial Board of the journal would accept symposia for publication with the decision to accept such symposia being made by the Editorial Board as a body.

A charter was established and the name of the journal was changed from *Cancer Chemotherapy Reports* to *Cancer Treatment Reports*. Under the direction of Dr. DeVita, the Director of DCT, the new charter established a new Editorial Board with Dr. Bruce Chabner as the Editor-in-Chief with 12 associate editors, and an Advisory Board of 15 prominent investigators from the extramural community.

As a result, in 1976 the three-part separately numbered system was abandoned and *CTR* began publication as a monthly journal. Currently, *CTR* considers manuscripts in eight major categories:

1. Full length manuscripts
2. Brief Reports
3. Letters to the Editor
4. Clinical Trial Summaries
5. Special Features (also guest editorials and commentaries)
6. Current Controversies in Cancer Management (solicited)
7. Meeting Reports
8. Mini-symposia

All submitted manuscripts are subject to review for scientific merit by at least two investigators in the area of treatment research under discussion and by the members

---

[1] Literature Research (*E. M. Sloane*) and Cancer Treatment Reports (*J. G. Baum*) Sections, Scientific Information Branch (*S. M. Hubbard*), Office of International Affairs; and Cancer Therapy Evaluation Branch, Division of Cancer Treatment (*R. E. Wittes*), National Cancer Institute, Bethesda, MD.

* *Reprint requests to:* Eleanor M. Sloane, Literature Research Section, Scientific Information Branch, Office of International Affairs, National Cancer Institute, Bldg 82, Rm B03, National Institutes of Health, Bethesda, MD 20205

of the Editorial Board. Based on reviewers' comments and the recommendation of the associate editor, a decision is made whether to accept or reject manuscripts and whether to request revisions. Approximately 50% of manuscripts submitted to *CTR* are currently accepted for publication and the time from acceptance to final publication is approximately 5 months. During the past several years, *CTR* has also published the proceedings of scientific meetings as intermittent supplements. Conferences on subjects of interest to the cancer research community are covered.

Initially the preparation of *CTR* was directed by the Publications Section of the Program Analysis Branch of the CCNSC, Chemotherapy Division. The Publications Section continued *CTR* publication under various administrative umbrellas in the DCT until November of 1980, when it became a section of the Division's Scientific Information Branch (SIB). In October 1983, the section became the Cancer Treatment Reports Section of an expanded SIB, in the Office of International Affairs, Office of the Director, National Cancer Institute.

The following tables show a ranked list of the most cited papers published in *CTR* (or *Cancer Chemotherapy Reports,* as applicable) from the years 1961 through 1980. The compilation was prepared by the Literature Research Section of the SIB. The tables are based on a list produced from the automated data base of *Science Citation Index* and includes all papers cited during the 20-year period, a total of 39,433 citations, ranging from 181 citations in 1961 to 6977 in 1980.

To make analysis of the citation records more meaningful, data were separated according to time of publication. Tables 1, 2, and 3 cover 1959-1964, 1965-1969, and 1970-1974, respectively. Separate tables (Nos. 4-7) are presented for each year from 1975 through 1978. Insufficient time has elapsed between articles appearing in 1979 and 1980 to allow enough information to determine ones likely to be more highly cited than others during that time. The data were analyzed to include, as completely as possible, all citations to a specific article regardless of errors in the format of the citation. A manual count was made from the 1981 cumulative *Science Citation Index* for the highest ranking articles.

While it is probably fair to equate citation frequency with the influence a paper has had, the precise meaning of these lists is open to discussion. Certainly the frequency of citation is affected by variables such as the general activity of research in a specific area, the interest in a given disease or treatment, and the intensity with which a certain therapeutic approach is being studied. The general shift from preclinical to clinical research over the past 25 years reflects the success of cancer chemotherapy in the treatment of advanced cancer and the current emphasis on clinical trials in oncology. Of interest is that as cancer chemotherapy has become more firmly established as an effective treatment modality, certain papers have become cited more frequently by the cancer research community over the years. The scientific contributions of Skipper, Schabel, and their colleagues have clearly stood the test of time. Other papers with remarkable staying power have dealt with the laboratory-clinical interface and with issues of statistical methodology. Not surprisingly, the most cited clinical articles deal with active drugs (cisplatin, doxorubicin, vindesine, AMSA), methods to exploit their activity, responsive diseases (lymphoma, testicular and ovarian cancer and small cell lung cancer), and concepts of interest in the formulation of treatment-related research questions. As such, *Cancer Treatment Reports* has served as an important forum for the presentation of new results and concepts in chemotherapy. We hope that in its second 25 years it will stimulate the development of other innovative approaches to cancer treatment.

TABLE 1.—Most cited papers published: 1959-1964

Author and title	Volume No.	Page No.	Year	Total No. of citations	No. of citations by yr									
					1961-1964	1965-1969	1970-1974	1975	1976	1977	1978	1979	1980	1981
Skipper HE, Schabel FM, Jr, and Wilcox WS. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemia	35	1	64	632	2	146	214	39	47	45	49	32	29	29
Rakieten N, Rakieten ML, and Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37917)	29	91	63	226	0	23	93	17	20	14	21	12	11	15
Handschumacher RE, Calabresi P, Welch AD, Bono V, Fallon H, and Frei E, III. Summary of current information on 5-azauridine	21	1	62	115	11	34	36	1	8	9	3	7	2	4
Elion GB, Callahan S, Bieber S, Hitching GH, and Rundles RW. A summary of investigations with 6-[(1-methyl-4-nitro-5-imidazolyl)thio]purine (B.W. 57-322)	14	93	61	97	3	24	32	8	4	5	3	6	9	3
Baker BR. The case for irreversible inhibitors as anticancer agents, an essay	4	1	59	94	33	29	12	1	4	3	3	2	4	3
Hewlett JS, Battle JD, Jr, Bishop RC, Fowler WM, Schwartz SO, Hagen PS, and Louis J. Phase II study of A-8103 (NSC-25154) in acute leukemia in adults	42	25	64	80	0	12	33	7	3	8	8	5	2	2
Skipper HE, and Schmidt LH. A manual on quantitative drug evaluation in experimental tumor systems	17	1	62	80	11	21	16	4	9	6	5	3	1	4
Frenkel EP, Skinner WN, and Smiley JD. Studies on metabolic defect induced by hydroxyurea (NSC-32065)	40	19	64	77	1	48	17	4	3	1	1	1	0	1
Frank W, and Osterberg AE. Mitomycin C (NSC-26980)—an evaluation of the Japanese reports	9	114	60	76	9	18	13	2	6	2	9	5	7	5
Armstrong JG, Dyke RW, Fouts PJ, and Gahimer JE. Hodgkin's disease, carcinoma of the breast, and other tumors treated with vinblastine sulfate	18	49	62	71	15	33	8	3	4	1	3	2	0	2

TABLE 2.—Most cited papers published: 1965-1969

Author and title	Volume No.	Page No.	Year	Total No. of citations	No. of citations by yr 1965-1969	1970-1974	1975	1976	1977	1978	1979	1980	1981
Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration	50	163	66	399	3	38	19	22	33	45	62	79	98
Skipper HE, Schabel FM, Jr, and Wilcox WS. Experimental evaluation of potential anticancer agents. XXI. Scheduling of arabinosylcytosine to take advantage of its S-phase specificity against leukemia cells	51	125	67	330	25	135	28	21	34	21	28	25	13
Freireich EJ, Gehan EA, Rall DP, Schmidt LH, and Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man	50	219	66	303	28	68	34	23	33	31	29	27	30
Di Marco A, Gaetani M, and Scarpinato B. Adriamycin (NSC-123,127): a new antibiotic with antitumor activity	53	33	69	197	5	73	19	14	26	16	14	16	14
Bodey GP, Freireich EJ, Monto RW, and Hewlett JS. Cytosine arabinoside (NSC-63878) therapy for acute leukemia in adults	53	59	69	143	0	52	10	11	16	15	12	17	10
Goldin A, Serpick AA, and Mantel N. Experimental screening procedures and clinical predictability value	50	173	66	130	34	50	3	11	7	10	6	3	6
Skipper HE, Schabel FM, Jr, and Wilcox WS. Experimental evaluation of potential anticancer agents. XIV. Further study of certain basic concepts underlying chemotherapy of leukemia	45	5	65	124	24	48	13	8	8	6	6	8	3
Adamson RH, and Fabro S. Antitumor activity and other biologic properties of L-asparaginase (NSC-109229)—a review	52	617	68	89	13	62	2	0	5	1	3	3	0
Wilcox WS, Griswold DP, Laster WR, Jr, Schabel FM, Jr, and Skipper HE. Experimental evaluation of potential anticancer agents. XVII. Kinetics of growth and regression after treatment of certain solid tumors	47	27	65	85	19	13	15	14	9	6	4	2	3
Evans JS, Gerritsen GC, Mann KM, and Owen SP. Antitumor and hyperglycemic activity of streptozotocin (NSC-37917) and its cofactor, U-15,774	48	1	65	82	13	37	5	9	4	4	3	3	4

TABLE 3.—Most cited papers published: 1970-1974

Author and title	Part	Volume No.	Page No.	Year	Total No. of citations	No. of citations by yr							
						1970-1974	1975	1976	1977	1978	1979	1980	1981
Geran RI, Greenberg NH, Macdonald MM, Schumacher AM, and Abbott BJ. Protocols for screening chemical agents and natural products against animal tumors and other biological systems	3	3(2)	1	72	640	28	45	68	96	81	115	110	97
Skipper HE, Schabel FM, Jr, Mellett LB, Montgomery JA, Wilkoff LJ, Lloyd HH, and Brockman RW. Implications of biochemical, cytokinetic, pharmocologic, and toxicologic relationships in the design of optimal therapeutic schedules	1	54	431	70	179	51	21	17	24	18	16	18	14
Simpson-Herren L, and Lloyd HH. Kinetic parameters and growth curves for experimental tumor systems	1	54	143	70	173	46	18	15	26	19	16	19	14
Mayo JG, Laster WR, Jr, Andrews CM, and Schabel FM, Jr. Success and failure in the treatment of solid tumors. III. "Cure" of metastatic Lewis lung carcinoma with methyl-CCNU (NSC-95441) and surgery-chemotherapy	1	56	183	72	125	16	16	21	28	18	11	9	6
Whitecar JP, Jr, Bodey GP, Freireich EJ, McCredie KB, and Hart JS. Cyclophosphamide (NSC-26271), vincristine (NSC-67574), cytosine arabinoside (NSC-63878), and prednisone (NSC-10023) (COAP) combination chemotherapy for acute leukemia in adults	1	56	543	72	123	31	18	17	18	16	8	11	4
Luce JK, Thurman WG, Isaacs BL, and Talley RW. Clinical trials with the antitumor agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388)	1	54	119	70	123	52	16	12	18	5	8	4	8
Wilson CB, Boldrey EB, and Enot KJ. 1,3-Bis(2-chloroethyl)-1-nitrosourea (NSC-409962) in the treatment of brain tumors	1	54	273	70	118	32	11	21	16	13	10	4	11
Walker MD, and Hurwitz BS. BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea; NSC-409962) in the treatment of malignant brain tumor—a preliminary report	1	54	263	70	101	29	11	12	12	14	7	9	7
Venditti JM. Treatment schedule dependency of experimentally active antileukemic (L1210) drugs	3	2(1)	35	71	99	34	14	8	11	8	12	5	7
Sandberg JS, Howsden FL, DiMarco A, and Goldin A. Comparison of the antileukemic effect in mice of adriamycin (NSC-123127) with daunomycin (NSC-82151)	1	54	1	70	99	43	14	13	9	6	6	7	1

TABLE 4.—Most cited papers published: 1975

Author and title	Part	Volume No.	Page No.	Year	Total No. of citations	No. of citations by yr 1975	1976	1977	1978	1979	1980	1981
Minow RA, Benjamin RS, and Gottlieb JA. Adriamycin (NSC-123127) cardiomyopathy—an overview with determination of risk factors	3	6(2)	195	75	154	2	10	20	41	31	25	25
Wasserman TH, Comis RL, Goldsmith M, Handelsman H, Penta JS, Slavik M, Soper WT, and Carter SK. Tabular analysis of the clinical chemotherapy of solid tumors	3	6(2)	399	75	149	0	9	30	37	23	32	18
Rosenberg B. Possible mechanisms for the antitumor activity of platinum coordination complexes	1	59	589	75	104	0	5	18	23	24	16	18
Di Marco A. Adriamycin (NSC-123127): mode and mechanism of action	3	6(2)	91	75	101	0	7	18	15	34	14	13
Davidson JP, Faber PJ, Fischer RG, Jr, Mansy S, Peresie HJ, Rosenberg B, and VanCamp L. "Platinum-pyrimidine blues" and related complexes: a new class of potent antitumor agents	1	59	287	75	95	2	10	13	19	14	19	18
Hill JM, Loeb E, MacLellan A, Hill NO, Khan A, and King JJ. Clinical studies of platinum coordination compounds in the treatment of various malignant diseases	1	59	647	75	94	4	5	9	19	28	10	19
Stoller RG, Jacobs SA, Drake JC, Lutz RJ, and Chabner BA. Pharmacokinetics of high-dose methotrexate (NSC-740)	3	6(1)	19	75	90	1	7	19	22	19	15	7
Gottlieb JA, and Drewinko B. Review of the current clinical status of platinum coordination complexes in cancer chemotherapy	1	59	621	75	89	2	8	16	15	21	12	15
Samuels ML, Johnson DE, and Holoye PY. Continuous intravenous bleomycin (NSC-125066) therapy with vinblastine (NSC-49842) in stage III testicular neoplasia	1	59	563	75	88	0	8	16	5	25	23	11
Pitman SW, Parker LM, Tattersall MH, Jaffe N, and Frei E, III. Clinical trials of high-dose methotrexate (NSC-740) with citrovorum factor (NSC-3590)—toxicologic and therapeutic observations	3	6(1)	43	75	74	2	7	15	20	13	9	8

TABLE 5.—Most cited papers published: 1976

Author and title	Volume No.	Page No.	Year	Total No. of citations	No. of citations by yr					
					1976	1977	1978	1979	1980	1981
Wiltshaw E, and Kroner T. Phase II study of *cis*-dichlorodiammineplatinum(II) (NSC-119875) in advanced adenocarcinoma of the ovary	60	55	76	114	2	12	18	36	22	24
Yagoda A, Watson RC, Gonzalez-Vitale JC, Grabstald H, and Whitmore WF. *Cis*-dichlorodiammineplatinum (II) in advanced bladder cancer	60	917	76	97	0	14	18	31	12	22
Myers CE, McGuire W, and Young R. Adriamycin: amelioration of toxicity by α-tocopherol	60	961	76	80	0	9	14	18	22	17
Arcamone F, Bernardi L, Giardino P, Patelli B, Di Marco A, Casazza AM, Pratesi G, and Reggiani P. Synthesis and antitumor activity of 4-demethoxy-daunorubicin, 4-demethoxy-7,9-diepidaunorubicin, and their β anomers	60	829	76	72	0	7	22	16	15	12
Comis RL. DTIC (NSC-45388) in malignant melanoma: a perspective	60	165	76	69	0	6	19	10	18	16
Jordan VC. Antiestrogenic and antitumor properties of tamoxifen in laboratory animals	60	1409	76	63	0	6	13	9	24	11
Lerner HJ, Band PR, Israel L, and Leung BS. Phase II study of tamoxifen: report of 74 patients with stage IV breast cancer	60	1431	76	56	0	2	15	8	19	12
Lloyd RE, Jones SE, Salmon SE, Durie BG, and McMahon LJ. Combination chemotherapy with adriamycin (NSC-123127) and cyclophosphamide (NSC-26271) for solid tumors: a phase II trial	60	77	76	53	0	8	14	10	13	8
Morgan LR, Jr, Schein PS, Woolley PV, Hoth D, Macdonald J, Lippman M, Posey LE, and Beazley RW. Therapeutic use of tamoxifen in advanced breast cancer: correlation with biochemical parameters	60	1437	76	51	0	0	17	10	14	10
Manni A, Trujillo J, Marshall JS, and Pearson OH. Antiestrogen-induced remissions in stage IV breast cancer	60	1445	76	49	0	2	16	8	15	8
Schabel FM, Jr. Nitrosoureas: a review of experimental antitumor activity	60	665	76	49	0	7	11	13	14	4

TABLE 6.—Most cited papers published: 1977

Author and title	Volume No.	Page No.	Year	Total No. of citations	No. of citations by yr 1977	1978	1979	1980	1981
Bunn PA, Jr, Cohen MH, Ihde DC, Fossieck BE, Jr, Matthews MJ, and Minna JD. Advances in small cell bronchogenic carcinoma	61	333	77	127	1	23	32	32	39
Wittes RE, Cvitkovic E, Shah J, Gerold FP, and Strong EW. *cis*-Dichlorodiammineplatinum (II) in the treatment of epidermoid carcinoma of the head and neck	61	359	77	79	0	13	18	23	25
Jaffe ES, Braylan RC, Nanba K, Frank MM, and Berard CW. Functional markers: a new perspective on malignant lymphomas	61	953	77	78	3	11	18	25	21
Eagan RT, Ingle JN, Frytak S, Rubin J, Kvols LK, Carr DT, Coles DT, and O'Fallon JR. Platinum-based polychemotherapy versus dianhydrogalactitol in advanced non-small cell lung cancer	61	1339	77	76	0	8	18	19	31
Cohen MH, Creaven PJ, Fossieck BE, Jr, Broder LE, Selawry OS, Johnston AV, Williams CL, and Minna JD. Intensive chemotherapy of small cell bronchogenic carcinoma	61	349	77	73	0	10	17	19	27
Lukes RJ, and Collins RD. Lukes-Collins classification and its significance	61	971	77	68	0	7	19	22	20
Anderson T, Bender RA, Fisher RI, DeVita VT, Chabner BA, Berard CW, Norton L, and Young RC. Combination chemotherapy in non-Hodgkin's lymphoma: results of long-term followup	61	1057	77	57	1	1	13	24	18
Goffinet DR, Warnke R, Dunnick NR, Castellino R, Glatstein E, Nelsen TS, Dorfman RF, Rosenberg SA, and Kaplan HS. Clinical and surgical (laparotomy) evaluation of patients with non-Hodgkin's lymphomas	61	981	77	54	0	2	9	28	15
Smolev JK, Heston WD, Scott WW, and Coffey DS. Characterization of the Dunning R3327H prostatic adenocarcinoma: an appropriate animal model for prostatic cancer	61	273	77	54	2	6	11	19	16
Norton L, and Simon R. Tumor size, sensitivity to therapy, and design of treatment schedules	61	1307	77	51	0	11	17	13	10

TABLE 7.—Most cited papers published: 1978

Author and title	Volume No.	Page No.	Year	Total No. of citations	No. of citations by yr			
					1978	1979	1980	1981
Von Hoff DD, Howser D, Gormley P, Bender RA, Glaubiger D, Levine AS, and Young RC. Phase I study of methanesulfonamide, *N*-[4-(9-acridinyl-amino)-3-methoxyphenyl]-(*m*-AMSA) using a single dose schedule	62	1421	78	44	0	3	19	22
Mathé G, Misset JL, De Vassal F, Gouveia J, Hayat M, Machover D, Belpomme D, Pico JL, Schwarzenberg L, Ribaud P, Musset M, Jasmin C, and De Luca L. Phase II clinical trial with vindesine for remission induction in acute leukemia, blastic crisis of chronic myeloid leukemia, lymphosarcoma, and Hodgkin's disease: absence of cross-resistance with vincristine	62	805	78	41	1	10	15	15
Billingham ME, Mason JW, Bristow MR, and Daniels JR. Anthracycline cardiomyopathy monitored by morphologic changes	62	865	78	39	2	9	16	12
Jaffe N, Frei E, III, Watts H, and Traggis D. High-dose methotrexate in osteogenic sarcoma: a 5-year experience	62	259	78	39	1	14	10	14
Bruckner HW, Cohen CJ, Wallach RC, Kabakow B, Deppe G, Greenspan EM, Gusberg SB, and Holland JF. Treatment of advanced ovarian cancer with *cis*-dichlorodiammineplatinum(II): poor-risk patients with intensive prior therapy	62	555	78	38	0	13	8	17
Dedrick RL, Myers CE, Bungay PM, and DeVita VT, Jr. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer	62	1	78	36	2	12	10	12
Tannock I. Cell kinetics and chemotherapy: a critical review	62	1117	78	31	0	5	11	15
Smith IE, Hedley DW, Powles TJ, and McElwain TJ. Vindesine: a phase II study in the treatment of breast carcinoma, malignant melanoma, and other tumors	62	1427	78	30	0	4	15	11

Contrasting Viewpoints on Cancer Drug Development: The Wooldridge and Richardson Reports

The two committees chaired by Drs. Wooldridge and Richardson had a great deal to say about the organization of the National Cancer Institute (NCI) as it impacted on research.

In this Appendix, the report of the Richardson Committee is published for the first time with a retrospective by Dr. Richardson. This preface is an attempt to give the reader a quick survey of the organizational changes at NCI, and to comment briefly on the outcome. In 1964, the Wooldridge Committee reviewed the intramural, extramural grant, and collaborative programs funded through contracts of the National Institutes of Health (NIH), including NCI's Drug Development Program. Their published report was critical of collaborative programs, particularly the Drug Development Program. In part, the criticism in the Wooldridge Report appears to have been a reaction to the special initiative and resources earmarked by the Congress for cancer drug development.

The diffuse organization of the NCI during its early years had the distinct advantage of allowing a high degree of interdisciplinary cross-fertilization between various components of the Institute, especially between its intramural clinical staff and those concerned with drug development.

Dr. James Shannon, who was then Director of the NIH, acted on the findings of the Wooldridge Report and, as a result, NCI Director Kenneth Endicott, in 1966, altered the organizational structure, incorporating the Cancer Chemotherapy National Service Center (CCNSC) program in a new chemotherapy division under the Institute's scientific director, Dr. C. Gordon Zubrod. This change was made to bring it into "conformity" with more traditional NIH programs. Within the Chemotherapy Division, separate programs were created headed by five associate directors: Experimental Therapeutics, Developmental Therapeutics (Drug Development), Medical Oncology, Cancer Therapy Evaluation, and the Baltimore Cancer Research Center.

Three other divisions, each with its own scientific director, were established in parallel to the treatment division: the Division of General Laboratories and Clinics headed by Nathaniel Berlin, the Division of Grants and Training headed by J. Palmer Saunders, and the Division of Cancer Etiology headed by Frank Rauscher. Under this new structure, the intramural Medical Oncology Program in the Chemotherapy Division was isolated from the specialties of surgery and radiotherapy, which now resided in the Division of General Labs and Clinics, as did NCI's Clinical Director. The extramural grant-supported clinical trials program, in the form of the Clinical Cooperative Groups (critical to the implementation of the fruits of the Drug Development Program), was now located in the Division of Grants and Training, artificially separated from contract-supported clinical trials, which resided in the Chemotherapy Division's Cancer Therapy Evaluation Program. There were barriers created to the free flow of communication and cooperation even within the Chemotherapy Division: the Toxicology Program, a vital resource for drug development, was part of the Experimental Therapeutics Program, while cancer-drug screening and formulation were in the Developmental Therapeutics Program.

To evaluate the response to the criticisms in the Wooldridge Report, a committee under Dr. Arthur Richardson was appointed in 1966. The Richardson Committee's conclusions with regard to the value of the collaborative program were diametrically opposite those of the Wooldridge Committee. They delivered a ringing endorsement of the past accomplishments and goals of the Drug Development Program. The report also expressed concern about separation of various components of the treatment program into different divisions of the NCI.

With the passage of the National Cancer Act in 1971, the NCI was reorganized once again, this time into four divisions, each given the responsibility for and named for a specific mission of the NCI, plus the new Cancer Control Program in the Office of the Director: the Division of Cancer Treatment (DCT) (formerly the Chemotherapy Division); the Division of Cancer Biology and Diagnosis (formerly the Division of General Labs and Clinics); the Division of Cancer Cause and Prevention (formerly the Division of Cancer Etiology); and a division responsible for both program and review of the NCI's extramural grants program, which also included its training programs and the expanded cancer centers program. Although a broader mission was implied by the name change for the treatment arm of the Institute, none of the resources required to fulfill this broader mission were transferred to DCT until 1975, when the Clinical Cooperative Groups were moved from the Grants Division to the Cancer Therapy Evaluation Program in the DCT and the intramural Surgery and Radiation Branches were transferred to the DCT as well. Then the DCT could, for the first time, command all the resources critical to drug development and the integration of chemotherapy with other modalities.

In 1978, to separate program management from review, the program management of grants was moved to each of the above divisions, depending on the research area encompassed by the grant, while the review of grants (and in 1981, the review of contracts) was made the responsibility of the Division of Extramural Activities. The final change was the merger of the former Control Division with the Centers Program, Training Programs, and the new applied prevention activities within a new division, the Division of Resources, Centers, and Community Activities.

The NCI now consists of five divisions: one responsible for program review (thus creating the critical separation of management of programs from their review) and four responsible for the direction of their individual programs. Each of the four program-oriented divisions now has all the appropriate tools at its disposal: grant programs, contracts, and intramural components.

Vincent T. DeVita, Jr, MD

Director, National Cancer Institute
Bethesda, Maryland

November 1, 1983

A Review of the Report of the 1966 Cancer Chemotherapy Collaborative Program Review Committee

Following Ehrlich's discovery, at the beginning of this century, that a chemical compound could selectively modify the course of syphilis, it became a common dream that a "magic bullet" could be found for most of the physical diseases which afflict man. From the beginning there were arguments and disagreements as to whether such discoveries should be sought by empirical screening of all compounds available, or whether one should devote the major efforts to studies on the fundamental nature of diseases to obtain the knowledge necessary to construct a rational basis for selection of substances for trial.

It is of interest that after the success and widespread use of arsphenamine, Ehrlich turned his attention to the problem of cancer. Since there was little basic information on the neoplastic process, his approach was naturally empirical. After a number of fruitless years he became discouraged. According to some accounts, he took what research money remained to him and set up a small laboratory for cancer studies, but over the entrance he placed a sign quoting from Dante which read: "All hope abandon ye who enter here."

The search for chemical compounds for selective action against diseases continued, especially against infectious diseases. For the most part progress came from the design of laboratory models of diseases and the empirical screening of compounds. The dramatic effects of sulfonamides, the antibiotics, and antimalarials raised the expectation that if only enough financial support were made available it would be possible to solve the cancer problem. For several decades the annual appeal of the American Cancer Society was based on the promise that a cure was just around the corner. The view that enough money could solve any problem was more widely held following Sputnik. This undoubtedly influenced the involvement of the federal government and the dramatic increase of funds made available to the National Institutes of Health (NIH). But it was largely on the premise that on some laboratory shelf or in some species of plant there existed Ehrlich's "magic bullet" for cancer, that the US Congress made a specific appropriation which initiated the cancer chemotherapy program in 1955.

It is not surprising that in a climate where financial support exceeded the requests of the scientific community, the possibility of some unwise allocation of resources could occur. The public, not realizing that a breakthrough was not on the horizon, increased the pressure to find a cure for cancer. In 1965, 10 years had elapsed and the chemotherapy program had expended a quarter of a billion dollars; yet there still was no cure in sight. As a consequence, there began to develop a feeling of disillusionment on the part of some legislators and the public about the effectiveness and efficiency of the cancer program.

As a result of general concerns about the growing health research support programs of the NIH and their administration, and following the directions of President Kennedy that a study be made, President Lyndon B. Johnson had appointed in 1964 a committee chaired by Dean E. Wooldridge to study the activities of the NIH and how that institution spent its approximately billion dollar budget. The final report issued by this committee in 1965 (the Wooldridge Report) was generally favorable to NIH, but expressed considerable concern about the NCI and its chemotherapy program and seriously questioned the expenditure of some $30 million on a single group of cancer chemotherapy collaborative projects. The committee advocated the appointment of an ad hoc committee to make a study of the Cancer Chemotherapy Collaborative Program and to recommend any necessary changes in direction.

The criticism of the collaborative programs raised questions, not only as to the general management of NCI's contract research, but brought into focus the question of the value of the empirical screening of chemical compounds versus a vigorous emphasis on basic research. It was in this climate that Dr. James Shannon, in 1965, adopted the recommendations of the Wooldridge Committee and appointed a committee to review the cancer chemotherapy program. The committee was purposely constituted of individuals of wide backgrounds and differing points of view. Although specifically charged with the task of exploring in depth the criticism made by the Wooldridge Committee, they were requested to explore all aspects of the cancer chemotherapy program. The diversity of the members made for lively and heated discussions. In the end, however, there was a remarkable degree of consensus as to what conclusions should be drawn. Among them were the following:

1. Chemical agents had been shown to control selectively at least some types of cancer.
2. While fundamental research may ultimately make it possible to select chemical compounds for study on a rational basis, such a rationale was not available in 1965; therefore, the empirical screening of compounds should continue.
3. Cancer should be considered as a complex disease or diseases and, if empirical screening were to succeed, a good deal of effort should go into a study of the screening procedures themselves so as to increase the chance that experimental results could be applied to man with greater success.
4. A cancer chemotherapy board should be appointed, to be composed of outside consultants who would be charged with the responsibility for continuous review of all aspects of the program. It was felt that such a board could meet many of the criticisms of the Wooldridge Committee by providing a mechanism for coordination of effort and better exchange of information between the various projects. Continuing review by such a board would also provide a means of evaluating new discoveries, whether from basic research or empirical screening. In this way the optimal utilization of resources could be made the basis of management decisions.
5. The Committee made a number of recommendations dealing with "housekeeping problems," most of which were already being implemented by the NCI staff.

In general it was the Committee's feeling that progress had been made in the conquest of cancer, and although the cost had been high it had been fully justified and should be continued.

The intervening years have proven the Committee correct in its conclusion that both basic research and empirical screening should be emphasized. A number of new effective chemotherapeutic agents have been discovered, and for the first time one has the feeling that basic research on the neoplastic process may be on the verge of providing the foundation for a more rational method for control of additional forms of cancer. Wisely, the modern-day prophets are not promising immediate results but are suggesting success by the end of this century. Let us hope they are overly conservative in their estimates.

Arthur P. Richardson, MD

Dean Emeritus, Emory University
School of Medicine, Atlanta, Georgia
Chairman of the Cancer Chemotherapy
Collaborative Program Review
Committee

August 12, 1983

Report of the Cancer Chemotherapy Collaborative Program Review Committee: The Richardson Report*

June 1966, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

INTRODUCTION

Organization of the Chemotherapy Program

The cancer chemotherapy program was initiated in 1955 and had two well defined objectives. The first was to develop an organized series of studies which would examine a broad range of candidate drugs in a systematic approach to solve elements of the cancer problem through the development of rational usage of new chemotherapeutic agents. The second was to determine if it is possible to demonstrate that a variety of chemical substances can effect the systematic cure of various cancers. The program now constitutes a significant segment of the NIH collaborative programs - approximately one-third. In the ten years of its operation it has expended approximately $214,000,000 and has established a complex system to 1) procure chemical agents, 2) screen them in animal tumor systems, 3) perform preclinical toxicological studies, and 4) conduct clinical evaluations of promising compounds.

The Wooldridge Committee Report

The relatively large sums of money expended by the NIH for these and other activities have prompted the formation of committees to inquire into the effective and economical management of the program. One of these committees was appointed by the President in 1964 and after an extensive review of the NIH operation, issued in 1965 a report entitled "Biomedical Science and its Administration - A Study of the National Institutes of Health."

The committee, headed by Dr. Dean E. Wooldridge, examined several aspects of the NIH programs, among which were 1) the largely grant-supported extramural program, 2) the largely contract-supported collaborative program, and 3) the intramural program. While the collaborative program was included in the study of the intramural program, because of the unique character of the collaborative program the recommendations concerning it were somewhat different. In general, the Wooldridge Committee Report was favorable to the extramural programs and concurred heartily in the general mission and objectives of the National Institutes of Health and the procedures that had been established to review and manage research grants. The committee found that the intramural research program of NIH was also of high quality but questioned the policy of undertaking university type research in a federal establishment. Concerning the collaborative programs the committee was critical. The review group found that a substantial fraction of the contract work in the cancer chemotherapy program was of lower scientific quality and showed evidence of inadequate central supervision.

Specifically, the group felt that 1) the operations of the Cancer Chemotherapy National Service Center have been less distinguished scientifically than the traditional research program supported by NIH and 2) the operations have been conducted without sufficient advice from the scientific community. The group stated further that "Even the work performed by essentially high-quality organizations (the majority) was less effective than it should be owing to lack of feedback, from the National Cancer Institute to the contractor, of test information he needed to do his job." Because of these findings the Wooldridge Committee recommended that an advisory group composed of a "suitable mixture of such technical management experts and medical research scientists is strongly indicated for performing a continuous review and appraisal function for each of the large collaborative programs."

---

*Copies of Dr. DeVita's Introduction, Dr. Richardson's Review, and the full Richardson Report may be obtained from: Scientific Information Branch, Office of International Affairs, National Cancer Institute, National Institutes of Health, Bldg 82, Rm 219, Bethesda, MD 20205.

TABLE 1
MEMBERSHIP OF THE CANCER CHEMOTHERAPY COLLABORATIVE
PROGRAM REVIEW COMMITTEE

Dr. Arthur P. Richardson (Chairman)
Dean, Emory University
School of Medicine
Atlanta, Georgia 30322

Dr. Paul Calabresi
Head, Section of Clinical
Pharmacology
Yale University Medical School
New Haven, Connecticut 06520

Dr. Thomas C. Chalmers
Chief of Medical Services
Lemuel Shattuck Hospital and
Professor of Medicine
Tufts University School of Medicine
Boston, Massachusetts 02130

Dr. Arthur C. Cope*
Camille Dreyfus Professor
of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Dr. Oscar Creech, Jr.
Chairman, Department of Surgery
Tulane University School of Medicine
New Orleans, Louisiana 70112

Dr. Harry F. Dowling
Head, Department of Medicine
University of Illinois
College of Medicine
Chicago, Illinois 60680

Dr. Franklin G. Ebaugh, Jr.
Dean, Boston University
School of Medicine
Boston, Massachusetts 02118

Dr. Louis S. Goodman
Department of Pharmacology
University of Utah College of
Medicine
Salt Lake City, Utah 84112

Dr. A. McGehee Harvey
Professor of Medicine
Johns Hopkins Hospital
Baltimore, Maryland 21205

Dr. Thomas H. Maren
Department of Pharmacology
and Therapeutics
University of Florida
College of Medicine
Gainesville, Florida 32603

Dr. Richard P. Mason
Vice President for Research
American Cancer Society, Inc.
219 East 42nd Street
New York, New York 10017

Dr. John V. Taggart
Professor of Physiology & Medicine
College of Physicians & Surgeons
of Columbia University
New York, New York 10032

Dr. J. Palmer Saunders
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014
(Executive Secretary)

*Deceased June 4, 1966

Organization of the Cancer Chemotherapy Collaborative Program Review Committee

The Director of NIH, in view of the recommendations of the Wooldridge Committee and the timeliness of the opportunity for a substantive review in depth, requested a group to make a study of the collaborative programs of the National Cancer Institute and to make recommendations to him concerning their continuation, expansion or contraction. A group was constituted under the chairmanship of Dr. Arthur P. Richardson and named the Cancer Chemotherapy Collaborative Program Review Committee (table 1), hereinafter designated throughout the report as the "Committee." It was given a broad charge and told to range as far afield as it desired to secure information upon which it could base its recommendations. The Committee first met in October 1965 and concluded its study in May 1966.

It was the purpose of the Committee to explore the techniques of study now in use in order to assess the reasonableness of the working hypotheses, the effectiveness of the program of studies developed to explore the reasonableness of the working hypotheses, and to determine the effectiveness of support programs, the integration of the clinical activities, and the decision making processes.

There are many indirect benefits which have resulted from the conduct of these studies by the cancer chemotherapy program, but presumably some, most, or all of these might have evolved through other support mechanisms and so cannot be considered to be the unique attributes of the chemotherapy program. Consequently, in the study and in the report, only those attributes of the current program of medical research which can be considered to be uniquely related to the chemotherapy effort were studied in any depth and are covered in the report that follows.

Finally, the Committee is aware of the rapid movement of science and the changing structure of support mechanisms which follow periodic program examinations. The Committee had the good fortune to examine the present program as it was in the process of modification subsequent to a review in depth in the spring of 1965 and after extensive discussions with advisory groups during the summer of the same year, culminating in a change of program emphasis presented to the National Advisory Cancer Council in the latter part of August 1965. Because of this it was possible for the Committee to assess the activity before, during, and after this program review.

Shortly before the formation of this Committee the National Cancer Institute had prepared for the August 1965 meeting of the National Advisory Cancer Council a comprehensive document embodying the history, current status and future objectives of the cancer chemotherapy program. Copies of this report ("The Cancer Chemotherapy Program" - a report to the August 1965 meeting of the National Advisory Cancer Council. To be published.) were made available to the Committee. The report, essentially factual in nature, served as an excellent point of departure for a study in depth of the chemotherapy program.

THE STUDY

The study program included:

1. Several meetings with the staff of the National Cancer Institute in order to obtain an understanding of the structure of the Institute, as to its operational attitudes and decision making processes and to explore in depth the philosophical basis of the program.
2. Discussions with a number of eminent scientists who have participated in the program for a number of years and who are located in the academic world.
3. Discussions with the authors of appendix four (collaborative research) of the Wooldridge Committee Report.

The Committee was subsequently divided into four subcommittees (table 2) to study some of the more important parts of the program in greater depth. The subcommittees considered:

1. Screening procedures
2. Pharmacologic aspects of the program
3. Contract supported research
4. Clinical trials

A series of project site visits was then undertaken, the sites selected to include:

1. Programs with more or less complete freedom from control by the National Cancer Institute. Both the project and the probable approach are largely initiated by the contractor.
2. Programs with less complete freedom. Generally speaking NCI determines project content but the contractor determines the approach to be used.
3. Programs with little freedom of action wherein NCI determines both the project and the approach by protocol.
4. Programs which are largely routine and wherein specified materials or services are closely defined.

The above categorization is based on a classification of all contracts by the chemotherapy program staff. Category 1 represents the largest dollar investment by NCI and the area of greatest flexibility of management and control. Every contractor in the category was visited. Contractors in the remaining categories were randomized and a sample of 15 percent was selected from each group studied. By chance in category 4 no routine screening laboratory was selected. The four such laboratories were randomized and one selected for study.

The subcommittee on clinical trials did not formally participate in the foregoing site visits, but concentrated on the grant-supported clinical collaborative groups. The subcommittee selected four of the less well known groups and, excluding chairmen, randomly selected four participants in each. These met with the subcommittees. Subsequently, the subcommittee met with three of the group chairmen. Finally, the chairman of each group was invited to submit in letter form his opinion of the accomplishments of his group and his estimate of the future needs of the clinical program.

The Committee also obtained comments on the program by letter from leaders in the field of chemotherapy.

TABLE 2
MEMBERSHIP OF THE VARIOUS SUBCOMMITTEES

Subcomittee on screening
  Dr. John V. Taggart (Chairman)
  Dr. Arthur P. Richardson

Subcommittee on pharmacology
  Dr. Thomas H. Maren (Chairman)
  Dr. Paul Calabresi

Subcommittee on contracts
  Dr. Thomas C. Chalmers (Chairman)
  Dr. Oscar Creech, Jr
  Dr. Richard P. Mason

Subcommittee on clinical trials
  Dr. Franklin G. Ebaugh, Jr (Chairman)
  Dr. Harry F. Dowling
  Dr. A. McGehee Harvey

FINDINGS AND RECOMMENDATIONS

General Considerations

The architects of the National Cancer Institute program in chemotherapy recognized that the field was difficult and complex, required broad support for many areas of science and technology of little interest to the academic scientist, and consequently would only yield, if at all, to a programmed effort that utilized the skills of industry in the program on a contract basis. It was also accepted from the beginning that, though essential, the activity would be both a costly and high risk type of expenditure.

The endeavor was both complex and empirical - characteristics of most programs aimed at the development of chemotherapeutic agents for wholly new systems. The particular difficulties presented by the cancer problem stem from the multiple nature of these diseases and the fact that the metabolic systems of tumor cells continue to defy simple biochemical differentiation from those of the parent cells from which they are derived.

Evidence is now available that one objective of the program has been achieved. It does appear that chemical agents can effect a cure of at least some cancer, e.g. choriocarcinoma and the acute leukemias of children. The other objective, the development of a series of effective therapeutic agents for general use in the more common tumors, has not yet been achieved.

The hypothesis upon which the cancer chemotherapy program is based, namely that a chemical substance can selectively eradicate cancer cells from the body seems, therefore, to have been sufficiently well-established to justify the basic concept and to warrant continued effort in the same direction.

General Quality of the Program

It is sometimes forgotten by scientists in other fields what complex problems are dealt with in the study of cancer, which is not a single entity but a bewildering group of diseases with widely differing characteristics. The single common denominator still is missing and until it is discovered, there is little choice but to continue with what is essentially an empirical approach. Small wonder then that the NCI chemotherapy program has posed difficult problems for those who would seek assurance that the sums of money appropriated for it are expended with maximum effectiveness and efficiency. If we feel this is a terrible problem we must also remember that cancer is a terrible affliction - so terrible indeed that it justifies the expenditure of a significant portion of the Nation's wealth on research aimed at its eradication. Because of this, the Committee views the efforts of the NCI staff in a humble manner, and can only attest to the single-minded devotion of the workers in the cancer field, both at NIH and at other institutions, to a field of study in which they encounter insurmountable obstacles with heartbreaking frequency.

It is the general feeling of the Committee that the overall quality of the program is good. Because of the diverse nature of the work supported the same standards of comparison cannot be used throughout. For example, the contract program involves work that is both basic and routine -

a contract can procure routine services on the one hand and highly original basic research on the other. The grant supported collaborative clinical trials program involves protocol studies involving comparisons of known chemotherapeutic agents in significant numbers of patients, but it can also support research on new drugs requiring considerable clinical sophistication. All of these elements are required in a total program, but it is not possible to judge each element of the program by the same standards.

The Committee finds that contracts that are primarily for original research such as the ones at Scripps Institute and the Hospital for Joint Diseases could compete successfully as project grant proposals. Contracts designed for a purely service function which do not involve research (and which probably would not be awarded as a research grant) seem to be conducted in a workman-like and conscientious manner and on these contracts in particular, there appears to be a remarkable amount of careful supervision by the staff of the chemotherapy program.

The large multi-phase contracts which had elements of both routine screening and exploratory research were difficult to evaluate because of the numerous activities being carried out. Contracts of this type require more intensive evaluation than the Committee was able to provide, and it is in this area that the review processes need to be improved.

The grant-supported clinical collaborative trials were not evaluated in detail but in general the Committee felt that the overall administration of the program by the NCI and the performance of the grantees has been of high quality. The review of the scientific merit of these programs should be by a special clinical trials panel composed of outside experts in the field of clinical research.

RECOMMENDATION

The present cancer chemotherapy program should be continued but the support and review mechanisms should be altered.

Need for a Chemotherapy Advisory Board

In the new organization of the National Cancer Institute the cancer chemotherapy program constitutes an important segment of the Institute's total activities. Until the reorganization no provision had been made for a formal body of outside consultants to review ongoing programs at the scientific level. For reasons referred to above it would be advantageous for the program to appoint a Chemotherapy Advisory Board to meet at least twice a year at the call of the Scientific Director for Chemotherapy. The body should serve as an active and continuing review group to provide advice to the Scientific Director on

1. The progress of the chemotherapy program and the directions for the future, and
2. Specific program areas proposed for contract support.

While it would be advisable to appoint as members people who do not have a significant role in the cancer chemotherapy program, it is recognized that it will be difficult to get a sufficient number of knowledgeable scientists without using those involved in the program; therefore, members of the advisory board should be selected solely on the basis of their scientific competence and of the relevance of their experience to the cancer program. It should be emphasized that the advisory board should not involve itself in specific contracts involving the procurement of facilities and service except insofar as these may bear on the program as a whole. But in the case of contracts for large sums of money involving multiple functions such as screening, toxicology and basic research, the advisory board should be in a position to provide advice to the Scientific Director on the scientific merit of the research elements of the contracts. The specific program review function may include, for example, the making of project site visits and discussions with appropriate NCI program officers. In order to conduct such reviews the board may wish to constitute several technical subcommittees utilizing other scientists as additional, outside members. In order to provide liaison and coordination with the National Advisory Cancer Council, it would be helpful to include a Council member on the advisory board.

RECOMMENDATION

A chemotherapy advisory board should be formed to provide the Scientific Director for Chemotherapy with scientific advice on

1) the progress of the chemotherapy program and the directions it should follow
2) specific program areas proposed for contract support

One member of the board should be a current member of the National Advisory Cancer Council.

Use of Outside Consultants

Subsequent to the final reorganization of the chemotherapy program the Committee found that an array of committees existed at all levels of the organization. While it is true that in certain areas, such as contract review, non-NIH members of recently constituted review committees were not in evidence, it was apparent that when project site visits are performed, outside consultants are employed and their reports are made available to the contract review committees. While this procedure certainly refutes any charge of lack of outside consultants, a letter or report is not quite the same thing as having such outside scientists actually sitting on the committee and participating in face to face discussions leading to a final recommendation. The value of outside consultants in advising Institute staff of the scientific qualities of ongoing programs and of newly proposed areas of research is well established. The Committee has the utmost respect and admiration for the scientific competence of the present staff of the chemotherapy program and there is no monopoly of wisdom possessed by the outside expert. Nevertheless, the Committee sees a real danger in failing to obtain and consider the advice of outside consultants. In the first place, people who are devoted to carrying out a specific program tend to overlook general considerations. The overview is a necessary check to a sometimes overenthusiastic specialization in a narrow field. Secondly, it would be advantageous to establish an advisory procedure that would effect a liaison with the scientific community at large. Finally, in terms of sheer input of new ideas it would be beneficial to obtain the advice of others than those who are actually engaged in the specific program.

RECOMMENDATION

> The Committee feels that although it cannot agree with the Wooldridge Report that the chemotherapy programs lack continuing scientific review by outside peers in the scientific community, it does feel that a more formal and visible advisory body should be created.

Coordination of Grants and Contracts

In the reorganization of the National Cancer Institute, the Committee noted that the chemotherapy program under its Scientific Director had no responsibility for the grant supported collaborative clinical trials program. Previously, the "collaborative" program had administered both contracts and clinical trials. Under the new organization the clinical trials will be administered by the Associate Director for Extramural Activities. It would be advantageous if both of these areas could be recombined in order to provide the necessary coordination for an informed judgment concerning future work. Since the office of the Scientific Director for Chemotherapy constitutes the main leadership in chemotherapy research in NCI, all research grants concerned with chemotherapy should be placed under this administration. There should be included in the organizational structure an assistant or an associate of the Scientific Director who would be charged with the responsibility of managing all cancer chemotherapy grants, including those supporting clinical trials; thus, when the Scientific Director for Chemotherapy presents his program to the Cancer Council for its information he, at the same time, will be able to present the grant program in the field of chemotherapy and should be in a position of making recommendations to the Council concerning priorities of payment.

RECOMMENDATION

> The administration responsibility for both grants and contracts in the area of chemotherapy should be placed in the Office of the Scientific Director for Chemotherapy.

Role of the National Advisory Council

The Committee recognizes the difference between contracts and grants insofar as the role of the Council is concerned. The Committee also recognizes that the Surgeon General may not award any grant unless it has been recommended for payment by a National Advisory Council. Similarly, it is recognized that the administrative officers of the National Cancer Institute have the sole responsibility for administering contracts and that this responsibility may not be delegated to a body of outside reviewers. Nevertheless, the Committee feels that these two areas of activity, namely grants and contracts, are really part of the same operation - that the objective of both mechanisms is to promote research that will conquer disease and it is felt that the Council, in order to be able to offer its advice to the National Cancer Institute and to make decisions on an intelligent basis, must have a complete knowledge of both areas of activity; therefore, the Committee believes that the National Advisory Council should be completely informed on all contract-supported activities and that the support of the National Cancer Institute in this area and its relationship to the proposed support to be given in the area of grants should be kept fully in front of the Council during its discussions. This mechanism should be publicized widely so there will be no misunderstanding about the role of the Council and its being kept completely informed at all times.

RECOMMENDATION

> The National Advisory Council should be provided with a coordinated report on progress and proposals in the areas of chemotherapy regardless of the mechanism of support.

The Contract Program

Many of the criticisms of the Cancer Contract Program revolve around the point that the amount of money expended is large compared with most other collaborative activities at the NIH. Many of the consultants interviewed by the Committee felt that an expenditure rate of $30,000,000 per annum was too high for the benefits likely to be produced in the program. Some would cut this, others would allow the money to remain but to change the type of work supported. Still others would place more emphasis on basic research and put more money into basic research programs supported by project grants. It seemed to the Committee that the National Cancer Institute, in its review of the collaborative program and in its moves toward reorganization and streamlining, has been well aware of the need for continual review of the size of the cancer collaborative program. From interviews with National Cancer Institute staff it was evident that progress was being made in reducing the size of on-going contracts and in eliminating those found to be unproductive. The recommendation given above for a chemotherapy advisory board should be extremely helpful to the cancer chemotherapy program in deciding on the correct size for a contract program and insuring that all contracts awarded are of high quality.

An implication of the recommendation for the establishment of an advisory board concerns the present direction of the cancer chemotherapy program. It has been indicated that contract supported investigations will be restricted initially to the exploitation of likely leads. At the present time there appears to be evolving a concentrated attack on the problem of acute leukemia, with an eventual extension to other leukemias, the lymphomas, and breast cancer in a step-wise process involving vigorous exploitation of promising leads. If this is the emphasis of the present chemotherapy program, the Committee wonders if the amount of money allotted is not too much for the support of a program in leukemia. One answer to this lies, of course, in the role of the chemotherapy advisory board. If the scientists serving on this board can represent the whole field of cancer, and, indeed, be representative of the entire scientific community rather than a particular area such as leukemia, the Committee feels that the program will evolve in a balanced fashion. The step-wise exploitation of leads in cancer chemotherapy seems to be a logical method of attacking a difficult problem, provided there is always an opportunity to support and exploit new leads in cancer chemotherapy, regardless of the particular disease entity concerned. The Committee feels that with the present leadership and with the advice and collaboration of a body of prominent outside scientists the chemotherapy program will go forward in good hands. The practice of dividing the support money between contracts and grants and of assigning grant money to the support of basic research and the development of new ideas and devoting contract money to the rapid exploitation of leads, seems to the Committee to be an excellent arrangement.

One topic that was discussed at considerable length not only by the Committee, but by consultants and contractors, was the specific nature of the activities covered by contract and their coordination by the staff of the chemotherapy program. There is no doubt that in terms of the specific contract objectives performance was generally good. The Committee feels that the National Cancer Institute is getting fair value for its money. It must also be said that there was considerable uncertainty about the quality of contract coordination exercised by the chemotherapy program staff. It was recognized early in the study that there were several distinct types of contracts administered in the program. A classification of these types is described in the "Study" section of this Report. Obviously, a contract for routine screening or animal supply is not managed in precisely the same way as a research project, and it is this difference that not only makes a resolution of the problem difficult, but causes confusion among outsiders who are unfamiliar with all the various aspects of the chemotherapy program. In general, the Committee felt that mission-oriented work such as routine screening, toxicology, synthesis, preparation of plant products, and animal procurement are ideally suited for a contract type operation. The present staffing structure of the chemotherapy program is well suited to administer such contracts. Free-ranging types of contracts which give the contractor considerable leeway in conducting his research pose difficult coordination problems. It is in this area, perhaps, that the greatest alarm is shown at an apparently incomplete "peer" review. By means of such contracts a considerable amount of money is devoted to research similar to that supported by the general grant program. Such research is not easy to control. One of the subcommittees found, for example, that in one well-funded contract of this nature certain segments of the contract were not being carried out according to the standard protocol. Upon further investigation it was discovered that the contractor was being permitted to continue the deviation because it was his judgment that the procedure was valid though admittedly different from the protocol. While the Committee did not examine the specific issues too closely, there was general agreement that if a protocol was available it should be followed and that if the contractor failed to conform to the provisions of the protocol then perhaps the entire contract operation at this facility should be critically re-appraised. This incident is cited not to harp on what may be a relatively minor matter, but to point up the difficulties involved in the coordination and control of large biomedical contracts which incorporate elements of considerable research latitude as well as routine, protocol-type efforts.

The varying degrees of scientific freedom afforded investigators supported by the contract mechanism suggested to the Committee that there should be different methods of handling those contracts which are not solely for the procurement of routine services or facilities:

1. Projects involving specific, independent research activities entirely.
2. Wide-ranging contracts that involve nearly every category as listed in "The Study" section of this report.

The Committee suggests that the purely research component of contracts be supported by means of the grant mechanism under which they can compete with other scientific proposals. In those contracts combining both routine protocol work and independent research activities, the present method of handling appears to result in an unsatisfactory situation concerning the overall scientific excellence. Where possible, the independent research elements of such contracts should be delineated clearly so that they can be easily subjected to a continuous review of their scientific merit by staff scientists and the chemotherapy advisory board. Such wide ranging contracts should also be limited in number and administered by a staff scientist who would insure that the research objectives of the contractor and those of the chemotherapy program are coincident. In no case should the administrative prohibition on the awarding of grants to profit making institutions be permitted to eliminate the "peer review" of the research components of such contracts.

RECOMMENDATION

1. The present direction of the cancer chemotherapy program should be continued.
2. Original research activities in non-profit institutions should be supported through the regular research grant mechanism.
3. Original research in profit making institutions, whether alone or in combination with more closely controlled activities should be delineated clearly and subjected to continual review by staff scientists and the proposed chemotherapy review board.

Problems of Communication in the Contract Program

A common allegation of contractors was the paucity of communication between the contractor and the National Cancer Institute. One contractor stated that there was great activity in the compilation of data but little in the evaluation of these data. Thus, it appeared that some contractors felt that the deficiency in "feedback" from the chemotherapy program was in quality not quantity. The Committee saw copies of brochures containing masses of data without any evaluative comments. Not only is it difficult to read such publications, but it is almost impossible to relate the material contained to any significant concepts unless some attempt is made to correlate it with earlier findings. The Committee got the impression that the efforts to analyze and evaluate the data provided in the chemotherapy program are far behind the rate of data production.

Another complaint of the contractors concerns a lack of communication with regard to the status of drugs following initial screening and toxicity studies. Almost all contractors expressed the view that they could do a better job if they had more of a feeling for the overall program accomplishments. The Committee feels that this is a reasonable point of view. More attention should be paid to reporting back to contractors follow-up data on the progress of drugs under test.

There also should be more scientific interchange between contractors' personnel and others working in similar or related areas.

RECOMMENDATION

> More effort should be devoted to speeding up research analysis and evaluation of data. The results of such evaluations, especially those referring to the progress of promising drugs, should be communicated rapidly to all participants in the chemotherapy program.

The Screening Program

One of the major activities of the chemotherapy program, and, indeed, the source of potential anti-cancer drugs is the program of screening maintained in various forms since the beginning of the Cancer Chemotherapy National Service Center.

Screening has never been carried out in a uniform fashion but has fallen into three broad categories:

1. The primary screen is maintained as a purely service function involving about eight different contractors. This type of screening is done strictly by published protocol after assignment of test compounds by the CCNSC to the contractors. The data which result from these tests are placed in a computer which is programmed to alert the staff to compounds found to be "active" in the primary screen. A fuller description of these procedures is given in Appendix Five.
2. A second type of screen is provided by contractors who furnish more detailed screening of compounds found active in the primary screen and of other materials of established interest. These contractors also participate in the development and assessment of new screening procedures. In general, such contractors have some latitude in initiating and modifying their test procedures.
3. A third type of screening is carried out by contractors and investigators who conduct relatively independent programs for the assessment of compounds of special concern to their own research or drug development programs.

There is considerable integration of all of these efforts by the chemotherapy program staff in order to select the appropriate screen for a given substance.

The Primary Screen

The first classification above, the primary screen, has been most frequently criticized in the past. Although over 100,000 materials have been screened for activity, the yield of new compounds or classes of compounds has been small (see Appendix Four, Table 1). Some of the criticisms of the screening program have been:

1. There is an empirical, and therefore, indiscriminate input.
2. The volume of materials processed is too great to permit an adequate follow-up and assessment by the limited staff of the chemotherapy program.
3. The predictive value of available animal experimental models for human disease is limited.
4. The recent restriction of the primary screen to two animal tumors, L1210 and the Walker 256 intramuscular rat tumor, may diminish its usefulness in uncovering new agents for use in the broad range of human neoplasms.
5. The quality of scientific performance by contract screeners has sometimes been unsatisfactory.
6. A primary screen provides only a crude index of anticancer activity. Potentially useful agents tested under a limited series of standard protocols often without regard for their peculiar physical, physiologic, and pharmacologic properties, may easily by discarded and not examined again.

The Committee found that in its present state of operation and considering the size of the staff, the screen is operating fairly well. The staff of the chemotherapy program exercises commendable care in accepting for testing only those compounds new to the program. With exceptions concerning biological data noted in the section on pharmacology, the staff does a remarkable job in assigning priorities for testing and assembling for the screeners the available information on solubility, stability and toxicity. There are also frequent consultations with contractors to plan the synthesis of new compounds.

The volume of input to the primary screen is large (some 18,000 samples per year) and this may well be taxing the capabilities of the present staff to perform their functions in the most efficient manner. This is especially true in the area of the appraisal of results. Some members of the

Committee felt that the broad-scale, indiscriminate screening of new compounds should be abandoned, but all agreed that there should be at least an appreciable reduction in the size of the input of compounds having a low probability for positive activity. It was not possible to arrive at a consensus on the means by which this could be accomplished, but one suggestion was that a sample accepted for screening should be of sufficient quantity to permit an adequate appraisal of its potential value. The fear was expressed that an inadequate sample inadequately tested might lead to a false negative.

When the CCNSC program was initiated the screening program was limited. Because of criticism of this limitation the screen was enlarged and eventually came to consist of more than a dozen different animal tumors. After a retrospective analysis of ten years of experience with various screens, the staff of the chemotherapy program decided to limit the primary screen to those tumors which responded favorably to a number of clinically useful compounds. The animal tumors responding best by this analysis are the mouse leukemia L1210 and the Walker 256 intramuscular solid tumor of the rat.

The Committee feels that this is a logical move which should prove useful in providing a more efficient search for active compounds. At the same time, however, the Committee feels that other screens should be maintained at least on a standby basis while additional experience with the two-tumor screen is being obtained. It is felt that the L1210 system is perhaps ideally suited to uncover leads for new agents against human leukemias (as well as against some other neoplasms), but that no acceptable models now exist for human solid tumors—by far the greatest problem in cancer.

RECOMMENDATION

1. In the absence of other dependable means for the discovery of new and useful agents, the primary screen should be continued but the volume of input to the screen should be reduced.
2. The decision to restrict the primary screen to two animal tumors appears to be a sound one for the immediate future, but it should be continually reviewed by the chemotherapy advisory board.
3. Increased efforts should be made to develop suitable animal experimental models of predictive value for the solid tumors of man.

Empirical Versus Rational Approach

The largely empirical nature of the primary screening process was a source of concern to many members of the Committee. Although it is recognized that current knowledge of the biology of cancer and the mode of action of chemotherapeutic agents is still too limited to support an entirely rational approach, the Committee feels that there should be an increasing emphasis on the acquisition of new knowledge through basic research. Toward this end, additional funds should be directed toward the individual investigator. The screening of new compounds by investigators or groups of investigators directly concerned with their development has the obvious advantages of interest, completeness, and careful attention to the peculiar pharmacological and toxicological properties of the drugs being studied.

RECOMMENDATION

In the continuing search for chemotherapeutic agents greater emphasis should be given to the acquisition of new information through basic research. Additional funds should be directed to individual investigators who can provide more rational bases for the continuing search.

Pharmacological Aspects of the Program

A weakness of the chemotherapy program is the lack of a sophisticated pharmacological approach in parts of the contract supported program. This is not due so much to a lack of awareness of this deficiency as to a serious deficiency in the number of available trained pharmacologists. With few exceptions, the toxicology being done under contract is in the hands of individuals with little or no formal training in pharmacology. This can result in changes such as the example, cited in a previous section of this Report, of a standard protocol not being followed properly. Other examples could be cited, but the general problem seems to lie in the inability of untrained people to "have a feeling" for drug action at all levels of study. In other words, when a drug is placed in a biological system there must be an awareness of the complex chemical and biological interrelationships that operate and that result, eventually, in a special kind of effect. Without such an awareness, the observation of these effects can become superficial and nonresponsive to the needs of a chemotherapy program. It would be beneficial for each major contract of the program to be under the surveillance of either a staff pharmacologist (as project officer) or a consulting pharmacologist. On certain large, complex contracts both types of individual could be usefully engaged. In the large contracts covering fairly complete programs, the contractor should be required to maintain a pharmacological group within the program. The pharmacologists in such a group should not only work on drug metabolism or enzymology, but should also participate and advise on the screening and toxicological aspects of the program and, additionally, should participate in planning for clinical studies.

RECOMMENDATION

1. Each major contract of the program should be under the surveillance of a pharmacologist, either from the National Cancer Institue staff as a project officer or from the scientific community as a consultant.
2. Each contractor engaged in extensive programs which range from screening to clinical studies should be required to maintain a pharmacological group within the program.

Problems of Communication in the Pharmacological Aspects of the Program

During the course of project site visits, members of the Committee encountered contractors who were uncertain of the activities of their fellow contractors. A specific complaint involved the lack of complete information about physical, chemical and biological characteristics of compounds forwarded for screening or for toxicological studies. This lack compelled the contractor to conduct studies on these characteristics himself. Although provisions for such studies are made in the standard protocols, it occurred to the Committee that if a given compound reaches the stage requiring studies of pre-clinical toxicology, there should be available data concerning the material's characteristics. If this information could be standardized and supplied to all contractors, there might be less unnecessary duplication of effort. There might also be less misunderstanding among contractors concerning the operation of the various segments of the contract program.

RECOMMENDATION

A central point in the chemotherapy program should be given the responsibility of maintaining and transmitting reliable physical, chemical and biological data on all compounds sent to contractors for additional study.

Training of Clinical Pharmacologists

In the area of clinical pharmacology, the Committee sees an even greater gap. The lack of trained clinical pharmacologists goes hand in hand with a serious deficiency in clinical pharmacological studies with new agents uncovered by the screen and deemed worthy of clinical trial. It is recognized that with the reorganization of the chemotherapy program and the inclusion of an Associate Scientific Director for Experimental Therapeutics, a great step forward has been taken, but there is little depth to the organization insofar as clinical pharmacology is concerned. Consequentially, few opportunities are available for the training of more clinical pharmacologists and it seems unlikely that the situation will improve in the near future. In view of this, Public Health Service Officers (of the caliber of NIH Clinical Associates) should be assigned to the few existing Clinical Centers that now have suitable programs of clinical pharmacology training. This training should include assignment as liaison clinicians with collaborative chemotherapy trial groups and with appropriate task forces. This approach would provide an attractive incentive to encourage academically oriented physicians to obtain the necessary training to fill this area of critical need. With such trained individuals available, greater emphasis could be placed on more extensive phase I and phase II studies stressing pharmacodynamics, dosage schedules and specific biological activity. This would lead naturally into the better planning of larger phase III studies.

RECOMMENDATION

Steps should be taken to train medical officers of the Public Health Service at existing Clinical Centers that now have suitable training programs in clinical pharmacology.

Collaborative Clinical Trials

General Considerations

Unlike the rest of the chemotherapy program the cooperative clinical trial groups are supported by means of research grants, commonly termed "programmed" grants to distinguish them from the traditional project research grants. The total grant money devoted to programmed grants approximates $5 million per annum and several hundred individuals participate through membership in 20 separate groups. Among these are Veterans Administration Groups which are supported by means of a transfer of funds rather than by research grants. Many of the groups have been operating ever since the inception of the program; others are fairly recent in origin. The Committee examined these groups with great interest and made some attempt to appraise the contributions that have been made. In general, considering the fact that this program was characterized by a rapid buildup, the over-all administration of it by the National Cancer Institute and the performance of the grantees have been of high quality. What problems have emerged have been met directly by the elimination of some groups and by the elimination of institutions not performing satisfactorily in terms of the protocol studies. The National Cancer Institute apparently anticipated by several years the current emphasis on patient care and physician training programs now evident in the Heart Disease, Cancer and Stroke Program. The cooperative clinical groups are good examples of this emphasis.

The clinical program has been criticized for 1) a relative slowness in accepting the principles of the controlled study, 2) a lack of experienced clinical pharmacologists and, 3) the use of rigid protocols which could lead to the premature discarding of potentially useful agents. The Committee recognizes the validity of these criticisms, but finds that the groups are making progress in overcoming them.

There have been many contributions by various groups. While lack of space precludes the inclusion of all contributions, a partial listing follows.

1. Methodology
   a. The development of criteria for conducting cooperative studies and for evaluating response or progress of disease.
   b. The development of sophisticated biometric techniques for evaluating the effect of drugs on cancer in human patients.
   c. The development and training of personnel in the discipline of clinical investigation in the field of cancer chemotherapy in humans.
2. The Introduction of New Drugs and Methods of Administration
   a. Use of L-sarcolysin in the treatment of multiple myeloma.
   b. Demonstration that Cytoxan is of use in the treatment of acute leukemia.
   c. Demonstration that the use of methotrexate intravenously twice weekly is more than twice as effective as oral administration.
   d. The combination of drugs acting independently may give at least summation effects, i.e. the effects of prednisone and vincristine are additive in acute leukemia.
   e. Intensive use of Vincristine, steroids, methotrexate and 6-mercaptopurine as initial therapy and in combination with supportive therapy such as platelet transfusions and white cell transfusions leads to prolonged remissions and possibly a cure in a small percentage of cases of acute leukemia in children.
   f. Mithramycin causes regression with prolonged remission in embryonal cell carcinoma of the testes.
3. An example of an important negative result is the demonstration that orally administered urethane has no effect on the course of multiple myeloma.
4. Ancillary Benefits
   a. The patient with cancer has been rescued from hopelessness, and in the case of far advanced cancer, neglect.
   b. Due to a marked increase in the interest of the physician and house staff, stimulated by the collaborative chemotherapy program, the gap between the discovery of new treatment techniques and their application to the patient has been significantly shortened.
   c. There has been a development of a sophisticated group of clinical investigators who can

promptly test any new drug of potential value in cancer in a sufficiently large number of patients with a wide variety of tumors to ascertain the drug activity with appropriate and valid statistical control.

Although the outstanding and well-recognized bottleneck is the acute lack of a sufficient number of really useful drugs for testing and evaluation, the Committee feels that there is much to be done in the way of finding better dosage schedules and routes of administration of existing drugs in cancer chemotherapy. The hard-won experience of the members of the clinical groups should be employed in a more extensive testing of various dosage schedules and routes of administration of known cancer chemotherapeutic drugs.

It was stated above that a deficiency in the number of clinical pharmacologists in the clinical trials program was one of the criticisms often encountered. This is a criticism that still holds true and is especially pertinent to some of the larger institutions which participated (see report of pharmacology subcommittee). There also seems to be a lack of communication among members of different collaborative groups within the same institution. For example, in those institutions housing two collaborative groups - one involving hematologist internists, the other, solid tumor surgeons, there was litttle evidence of communication between the groups. It would be beneficial to the clinical trials program as a whole if all groups were to communicate freely and to keep informed of the others' activities.

RECOMMENDATION

1. There should be a more extensive testing of various dosage schedules and routes of administration of known cancer chemotherapeutic drugs.
2. More emphasis should be placed on clinical pharmacology in the collaborative clinical trials program.
3. A concerted effort should be made by the National Cancer Institute to effect better communication among members of the various clinical groups.

Administration of Clinical Grants

The Committee noticed that there appeared to be varying policies covering the funding of the different groups. There may be excellent reasons for these differences, but it is felt that groups of a similar nature should be funded in a similar fashion. Institutions involved in across the board studies, such as the Acute Leukemia Groups A and B, the Southeastern Group, the Southwestern Group, and those groups exclusive of the Surgery-Adjuvant Groups, should be funded in a uniform manner with individual grants being made to each institution. It was noted also that some groups applied for renewal every two years while others applied every three years. It would add stability and better performance if more long term funding were offered to those groups which have performed well in the past.

In a recommendation made earlier in this Report it was suggested that grants for the cooperative clinical trial groups be placed under the leadership of the Scientific Director for Chemotherapy. In doing so the National Cancer Institute should insure that the evaluation of these grants would reside in a study section type of committee with rotating membership. The members of this review panel should be individuals with special competence in the field of clinical investigation and drug evaluation.

RECOMMENDATION

1. Similar groups should be funded in a uniform manner with individual grants being made to each institution. Groups that have performed well in the past should be given long term funding.
2. A special review panel should be established to review the grant supported clinical collaborative groups. The groups supported by transfer of funds to the Veterans Administration should be reviewed by the same panel.

Longitudinal Studies

It was quite apparent to the Committee in its discussions that an optimal form of cancer chemotherapy research is one in which the whole process of study from the screen to the clinic is in the hands of a coordinated research team which has an interest in a particular class of compounds. People with such interests are alert to the vagaries of screening and pharmacological studies and sometimes will have the enthusiasm to overcome initially negative results and to try different dose forms and schedules to bring a compound to an eventual successful trial. In the field of cancer chemotherapy research there is another screen that operates before a compound is ever submitted to the Cancer Chemotherapy National Service Center. Any investigator who develops a compound which he thinks might be active and who has immediate access to a longitudinal screen will take the compound there for trial in this screen and if any encouragement is obtained at all, will attempt to become associated with the compound all the way through the screen to the clinical trial. It seems to the committee that this sometimes operates to prevent likely looking compounds from entering the CCNSC program. There is no objection to this, but CCNSC should recognize that sometimes longitudinal studies offer an attraction that the diverse but distant activities of the CCNSC cannot offer.

RECOMMENDATION

> The National Cancer Institute should explore the feasibility of increasing the support in depth of established investigators who have the capability of operating well coordinated and controlled longitudinal studies.

The Task Force

One of the unique features of the chemotherapy program is the body of advisors termed a "task force." While several exist, by far the most important is the one devoted to acute leukemia. This is one device through which the Scientific Director for Chemotherapy attempts to coordinate the activities of his program - from the synthesis or purification of a new drug to its final trial in a clinical study. These groups meet frequently enough to manage an excellent rapport among all segments of the scientific groups working on the problem of acute leukemia. It will be noted that the membership of the groups ranges all the way from basic laboratory scientists to active clinicians. The cross fertilization of ideas alone makes this an attractive arrangement and there is little doubt that the mechanism represents an efficient method for tackling a specific problem. The Committee feels that this is an excellent method of procedure and feels that it should be continued and carried over to other cancer areas when the state of knowledge is such as to warrant an all out attack on the problem.

RECOMMENDATION

> The Committee commends the National Cancer Institute for its utilization of the task force approach and recommends that the concept be extended to other areas of cancer when it becomes propitious to do so.

ACKNOWLEDGMENTS

The Committee wishes to acknowledge the excellent cooperation of the staff of the National Cancer Institute, the contractors and grantees of the chemotherapy program and the eminent scientists who met with the Committee to discuss research in cancer chemotherapy. Thanks are due also to Dr. Robert Edmund Handschumacher, Department of Pharmacology, Yale University Medical School and Dr. Paul Talalay, Department of Pharmacology, Johns Hopkins University School of Medicine, for their valuable help on project site visits for the Committee. The Committee wishes particularly to express its appreciation to those scientists who took valuable time to send written statements concerning major problems of the field. One of the members of the Committee, Dr. Louis S. Goodman, underwent surgery shortly after the Committeee had begun its study and was unable to attend more than one meeting. However, he was able to review all of the material available to the Committee as well as the final draft of this Report and he has expressed his concurrence in the findings and recommendation. Finally, the Committee wishes to thank Mrs. Hazel Shankel for her effectiveness in arranging meetings, project site visits and arranging for suitable background material without which the Committee's work would have been difficult.

Also part of this report, but not reprinted here, are the following appendices.

Appendix One - Chronology
Appendix Two - Subcommittee Reports
  A. Subcommittee on Screening
  B. Subcommittee on Contracts
  C. Subcommittee on Pharmacology
  D. Subcommittee on Clinical Trials
Appendix Three - Structure and Fiscal Data
Appendix Four - Useful Drugs Uncovered by CCNSC Screen
Appendix Five - Description of Activities of the Drug Development Branch and the Drug Evaluation Branch, CCNSC

☆ U.S. GOVERNMENT PRINTING OFFICE: 1984- 421-140:107

A Publication of the National Cancer Institute

NIH Publication No. 84-22